Chapter 1
Bacterial Stress Responses

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Abstract The bacterial stress response is an early-evolved system of protein-folding proteins, both molecular chaperones and protein-folding catalysts, linked to appropriate transcription factors for generating appropriate cellular responses to external and internal stress. Two key requirements of this vital stress response system are *robustness* and *resilience.* Most of our understanding of the bacterial stress response comes largely from study of the K12 laboratory strain of *E. coli.* The essential elements of the cytoplasmic stress response are the protein pairs: chaperonin 60/chaperonin 10 and DnaK/DnaJ whose mechanisms of action are distinct but which work together to maintain cytoplasmic proteostasis. Other cytoplasmic stress proteins include the small heat shock proteins and bacterial Hsp90. The other major cell volume in bacteria is the periplasmic space which, in contrast the cytoplasm, has an oxidising environment. This requires another set of stress proteins including the protein disulphide isomerases and the peptidylprolyl isomerases. Essential to our understanding of the bacterial cell stress response is the realisation that it is a *biological control system* whose overall role is to enable prokaryotes to cope with any stresses that the environment throws at them – such as the infectious process. It is unclear how the moonlighting actions of certain of these proteins integrates with their protein-folding/stress relieving actions.

1.1 The Concept of the Stress Response

If we define a stress as being any event that compromises the normal function of an organism, it is obvious that there are two basic strategies that can protect against that stress. One is to have a pre-existing capacity to resist the stress. This works, up to a

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point, but its maintenance requires the continuous use of resources that could be devoted to some other activity. The other is to detect the stress and respond to it, perhaps by moving to a less stressful location, or by changing properties so that the stress is no longer so damaging. This also requires resources, but they are only used when needed. The two strategies can be described as *robustness* and *resilience*. Organisms have to find the correct balance between the two. Those that fail to do so will not reproduce, and so will be selected against. Modern day organisms are examples of systems that have evolved to have an optimal balance between efficiency on the one hand, in terms of being able to use resources (energy, food, and the environment in which they live) to pass on their genes to the next generation, and robustness and resilience on the other, in being able to deal effectively with any stresses that they are likely to encounter.

The study of how bacteria deal with stress can be very enlightening in this regard. Bacteria are simple compared to eukaryotes, with most bacteria having much smaller genomes than most eukaryotes. Bacterial genomes are dense with genes, and low in features like repetitive DNA. This is not because of their need to replicate quickly but because in the absence of selection, genes are easily lost by deletion (Mira et al. 2001). In looking at a bacterial genome we are looking at a fairly minimal set of genes for life, and genes that are involved in helping the organism resist stress will have been strongly selected for. Add to this the well-known experimental reasons for working with bacteria, and the fact that some of them cause infections, and it is evident that the study of bacterial stress responses will pay dividends in areas well beyond microbiology.

Simple principles must apply to any resilient system that is invoked only when the relevant stress is detected. First, the organism must be able to detect the stress and distinguish it from other stresses. Second, this detection must be linked in some way to the response, as it is no use being able to detect a stress unless the consequence is the mounting of a relevant response. Third, the response has to be appropriate to the particular stress. For example, there would be no point in responding to a dangerous down-shift in pH by turning on the genes that are required to deal with an osmotic shock, either through a failure of the detection system, the incorrect linkage to the response, or the faulty nature of the response itself. Fourth, the response must be switched off when the stress is no longer a problem, since not to do so would lead to a non-competitive reduction in efficiency for the organism overall. All of these features – detection of the stress, transduction of the signal, response to the stress, and termination of the response – are exemplified in the many bacterial stress responses that have been investigated, although the fourth point is less well studied that the other three.

1.2 Methods and General Principles

Much research on bacterial stress responses has focussed on describing the changes in gene and protein expression following stress, and evaluating the significance of these. In the pre-genomic era, the two major experimental approaches to this were

to identify proteins whose synthesis was induced or elevated following stress, and to find mutants that failed to cope with the stress, often by tagging followed by cloning of the genes involved, and investigate their regulation and the consequences of their absence. The focus has now shifted to more high throughput methods, principally proteomics and transcriptomics. Systems biology approaches that use 'omics data to build models of circuits and pathways are beginning to lead to a more holistic view of how stress responses are organised and integrated with other aspects of the biology of the organism, but we are still a long way from having organism-level models that enable us to directly link the overall stress response to a quantifiable measure of fitness. However, even having a list of the components and an idea of their individual properties is a good place to start. Consequently the armoury of molecular biology and biochemistry has been brought to bear on organisms and on purified proteins that are induced or up-regulated in response to stress.

The way that a bacterium responds to a stress depends on its growth medium, the level of the stress, the time over which the stress is applied, the precise nature of the strain used, and a myriad of other factors. One of the most important of these is probably which other stresses are also being applied at the same time, and investigations are now moving towards the recognition that at any time (and in particular in the bacterium's natural environments – which may be our gut, our blood stream, or our macrophages) a bacterium will be responding to multiple different stresses simultaneously. A successful bacterium like a pathogen must have evolved systems to simultaneously integrate the input of many different signals (temperature, nutrient availability, external pH, osmotic pressure, presence of external signalling molecules, oxygen tension, and many more) and respond to them in such a way as to maximise the bacterium's chance of survival. We are still only scratching the surface of understanding how bacteria do this. In many cases we do not understand exactly what damage these stressors can do to the cell, which means we often do not fully understand why some aspects of the responses that we see to these stressors are good ones for the organism to have.

Another key experimental variable is the choice of experimental organism. The largest amount of work on stress responses in bacteria has been done on various strains of *Escherichia coli* K12, the standard laboratory workhorse. Much that has been learned from *E. coli* can be applied to other bacteria, but this must be done with caution, as stress responses can show significant variation even between different strains of the same species. Although some responses, such as the heat shock response, are very well conserved, others are very species specific. *E. coli* for example is well adapted to survive low pH, and has several inducible systems that enable it to do this that are not present even in other enteric bacteria (Foster 2004). Also, the mechanism of regulation of the same response may vary significantly between different species, as is the case with the bacterial heat shock response (HSR). There is also abundant evidence that components of stress responses such as the HSR can, in different bacterial species, evolve to take on new cellular functions. This important point is discussed further below. Thus, while much of what is discussed below is based upon studies in laboratory strains of *E. coli*, it must be borne in mind that extrapolation is risky.

There are many different ways in which the proteins of a particular stress response enable the bacterium to deal with the stress. Bacteria may engage in damage-limitation, where they induce proteins that deal with the consequences of the stress, perhaps by repairing DNA damage, or (as will be discussed in detail below) refolding or degrading misfolded proteins. They may attempt to decrease the level of the stressor, for example by the induction of superoxide dismutates or catalases to deal with high levels of damaging superoxide or peroxide. They may alter their properties, for example by changing the proportion of saturated and unsaturated fatty acids in the membrane to make it more fluid in response to chilling. In the case of stress brought about by nutrient limitation, they are likely to improve their ability to scavenge for, or to import such nutrients, by the production of compounds such as siderophores for scavenging iron or the up-regulation of specific transporters, or they may switch on alternative metabolic pathways. Many stresses invoke more than one of these strategies. Behavioural responses are also common: bacteria can swim towards nutrients and away from stressors, and if all else fails they can simply sit the stress out, for example by forming spores or other highly robust forms.

Having made these general introductory points, I will now discuss the bacterial heat-shock response. It is an excellent example of a well-studied response where the components are understood and their properties have been studied in detail, the signals for inducing it are fairly clear (though probably not all documented), there are very interesting differences between different bacteria, and there is, of course, abundant evidence, discussed in the rest of this volume, that many of the proteins in this response have multiple moonlighting features.

1.3 The Heat-Shock Response

The heat shock response (HSR) is what it says on the tin: a response to heat. This does not mean that it is only induced in response to heat, but heat is a biologically relevant parameter which is easy to vary experimentally, and hence the HSR is probably one of the most studied of all stress responses. First described (Ritossa 1962) as a phenomenon that induced "puffing" in the polytene chromosomes of salivary glands of Drosophila that were moved from their normal growth temperature to 37 °C, (for more details see Chap. 2) it had been studied for nearly two decades before being looked at in any detail in *E. coli* or other bacteria (Neidhardt et al. 1984). The HSR occurs at temperatures a few degrees above the normal optimal growth temperature of the organism concerned, so organisms from the polar regions can show a HSR at 10 °C, whereas those that normally bask in hot springs require temperatures of nearly 90 °C to induce their HSR (Clark and Peck 2009; Trent et al. 1990). It consists of the induction or up-regulation of synthesis of a set of proteins, many of which are very highly conserved across kingdoms, and many of which have been shown to have key roles in cell growth, including in some cases at normal temperatures as well as under heat shock conditions. The predominant role of these proteins is in protein quality control – aiding the folding of some cellular proteins, and

degrading those that fail to fold correctly. The high degree of conservation of the response across all domains of life in terms of its components and the conditions that induce it, shows that this is a very ancient response indeed, likely having been present in some form in the last universal common ancestor.

Heat shock genes are induced by many treatments other than heat, including desiccation, osmotic stress, ethanol, and heavy metals. Even the original paper by Ritossa noted that the metabolic uncoupler DNP could induce heat shock. This shows that the response is not simply due to an increase in temperature, and any hypothesis concerning the mechanisms whereby the HSR is induced has to take account of this.

1.3.1 What Is the Role of the HSR?

The role of the HSR is best deduced from studies on mutants in which it does not take place. Such mutants exist in *E. coli*. In this organism, the HSR is induced by an expansion in the repertoire of promoters that are recognised by RNA polymerase, caused by the increased presence in the cell after heat shock of a particular RNA polymerase sub-unit, responsible for promoter recognition, called σ^{32} (Guisbert et al. 2008). A mutant that lacks the gene for this RNA polymerase sub-unit cannot mount a HSR. Such a mutant has two striking phenotypes. The first is that it is extremely temperature sensitive, failing to grow at temperatures above 20 °C, significantly lower than the optimum for *E. coli* of 37 °C (Zhou et al. 1988). The other is that a large number of its proteins are present as insoluble aggregates (Gragerov et al. 1991). From this it can be deduced that the HSR is something of a misnomer, since its expression is needed for growth even under non-heat shock conditions. It can also be deduced that a key role of the HSR must be to ensure that proteins do not aggregate, but either reach their correct soluble form, or be degraded if they fail to fold correctly.

1.3.2 Identification and Roles of the Key Components of the HSR

Although the members of the heat shock regulon can be identified by studies of the transcriptome or by over-production of σ^{32} (Richmond et al. 1999; Nonaka et al. 2006), such studies do not give any information about the roles or relative importance of these *members*. However, genetic experiments in *E. coli* have already provided this information. Because σ*rpoH* mutants of *E. coli* are highly temperatures sensitive, it is easy to select for mutants in these strains that have recovered the ability to grow at temperatures above 20 °C. Such mutants showed over-expression of a protein called GroEL, which was already known to be a heat shock protein (Kusukawa and Yura 1988). The temperature at which the mutants grew correlated well with the

extent to which the GroEL protein was over-expressed. No mutants were found when selections were done above 40 °C, but if a second round of selection was done on strains that already over-produced GroEL, such mutants could now be found. These all showed elevated expression of another heat shock protein called DnaK. Thus, the severe defect in growth caused by loss of the *rpoH* gene can be overcome by increased expression of GroEL and (at higher temperatures) DnaK, showing that of all the proteins of the HSR, these two are particularly important.

Over-expression of these two proteins is now known to be accompanied by the over-expression of other proteins encoded by the same operons. In *E. coli*, these are called GroES (co-expressed with GroEL) and DnaJ (co-expressed with DnaK). The central roles of these proteins was confirmed by further experiments done on strains containing *rpoH* mutations. The introduction of plasmids over-expressing either GroES and GroEL, or DnaK and DnaJ, largely prevented the protein aggregation seen in the cytoplasm of these strains (Gragerov et al. 1991, 1992). These studies showed that expression of both partner proteins was required for preventing aggregation: GroEL was ineffective without GroES, and DnaK was ineffective without DnaJ.

Thus there are two separate protein machines in the bacterial cell that are required for proteins to become fully soluble, even under normal growth conditions, and particularly after heat shock: the GroES/GroEL pair, and the DnaK/DnaJ pair. At the same time that these experiments were being done, related experiments in other organisms and using *in vitro* components was also studying the roles of homologues of these proteins, and the term "molecular chaperone" was coined for them in 1988 (Hemmingsen et al. 1988). Molecular chaperones were defined as proteins whose action was required for other proteins to attain their fully folded form. With these discoveries a new field of biology was born, and over the last 25 years an enormous amount of work has been done to document the cellular roles, mechanisms, and structures of many molecular chaperones (see also Chap. 2 for more discussion of protein folding and molecular chaperones). Some of the key findings in this field, which are relevant to the present volume, are summarised below. But first, the author will take a brief look at the way in which the HSR is regulated, since this may be relevant for the expression of these proteins in infecting bacteria.

1.3.3 The HSR Is Mostly an Unfolded Protein Response

Heat shock is generally studied simply by raising the incubation temperature of cultured cells, but as noted above, many other treatments can induce the HSR without any change in growth temperature. What is the signal (or signals) that cells detect in order to mount a HSR? The answer appears to be that a key signal is the presence of unfolded proteins in the cell. Several lines of evidence support this hypothesis. For example, the HSR can be induced in *E. coli* at 37 °C by the presence of amino-acid analogues (Goff and Goldberg 1985), by expression of proteins that fail to fold correctly (Parsell and Sauer 1989), and by the presence of precursors of

secretory proteins (Wild et al. 1993). This last point probably explains of the ability of ethanol to induce the HSR, since treatment of cells with ethanol inhibits protein secretion (Chaudhuri et al. 2006).

There are very plausible mechanisms that explain how accumulation of unfolded protein leads to the induction of the HSR in bacteria (reviewed in detail in Guisbert et al. 2008). The details of these vary between bacteria. In *E. coli*, accumulation of unfolded protein leads to stabilisation of the σ^{32} sub-unit of RNA polymerase. σ^{32} is normally very unstable, being degraded mostly by the FtsH protease, and the action of DnaK is needed for this degradation. However, DnaK also binds to unfolded proteins, so the model proposes that when unfolded proteins accumulate in the cell, DnaK is saturated by binding these proteins and hence is no longer available to bind σ^{32}. σ^{32} is thus stabilised, binds to RNA polymerase, and the promoters of the heat shock genes become activated. This model is well supported by experimental data, and a mathematical model of key elements in this system gives good agreement with experimentally determined events (El-Samad et al. 2005). In many other bacteria, the mechanism of regulation of the heat shock genes is different (repressor-mediated systems are more common that the alternative sigma factor system found in *E. coli*) but good evidence, including mathematical modelling, also supports an unfolded protein titration model in these cases as well (Narberhaus 1999; Inoue et al. 2012).

Thus, we have a mechanism for detection of the stressor, for transduction of this information, and we know the cellular role and the key components of the response. What do we know about the detailed roles of these key components?

1.4 The Cellular Networks That Assist Protein Folding in Bacteria

A concept that has gained increasing prominence in recent years is that of proteostasis (protein homeostasis) – the concept that cells have systems in place to maintain an overall balance of folded protein, that operate all the time but become particularly important under conditions that perturb protein folding (Balch et al. 2008). The key protein complexes identified by the genetic approaches above are integral parts of the proteostasis network in *E. coli* (and other bacteria), but they have somewhat different roles. In the next section, the roles of these two protein complexes, plus other components of the HSR network, will be discussed.

1.4.1 The Chaperonins

The GroEL protein is the *E. coli* representative of a class of proteins, the chaperonins, that have been shown to be essential in all domains of life (Fayet et al. 1989; Stoldt et al. 1996; Kapatai et al. 2006). The generic name for these proteins is Cpn60 proteins (for chaperonin 60, as the molecular weight of a monomer is approximately

60 kDa), and the small proteins that interacts with them are called Cpn10 proteins. Cpn60 and Cpn10 proteins are highly conserved in sequence and function; for example, the homologue from human mitochondria can complement for loss of the GroEL protein in *E. coli* (Nielsen et al. 1999).

Cpn60 and Cpn 10 proteins are abundant in bacteria, even in cells which have not been heat shocked. In a list of detectable proteins by relative abundance in *E. coli,* GroEL ranks in the top 3 %, or the top 1 % if the highly abundant ribosomal proteins are excluded; GroES is just outside the top 10 % (Ishihama et al. 2008). The Cpn60 proteins are usually found in a complex with a striking structure, normally consisting of two rings of sub-units, stacked in a "double doughnut" (Braig et al. 1994). In prokaryotic homologues, which are also found in chloroplasts and mitochondria, there are seven sub-units in each ring. Each ring of sub-units contains a central cavity where protein folding occurs. The past two decades have seen a very significant research effort into understanding the mechanism of these "protein folding machines", and although there is still controversy over some of the details, the basic reaction cycle has been thoroughly described (and is recently reviewed in Yébenes et al. 2011; Horwich and Fenton 2009; Horwich et al. 2007). The essence of the reaction cycle is that it involves binding of unfolded or partially folded client protein to the open end of one ring of the complex, followed by its displacement into the cavity inside the ring by the binding of Cpn10. Its subsequent ejection from the cavity requires the binding of another client protein and Cpn10 to the opposite ring. The cycling between binding at the two ends is mediated by the binding and hydrolysis of ATP.

There are several steps in the cycle that may aid protein folding. None of these involve Cpn60 providing any steric information to the folding client protein, indeed the role of Cpn60 proteins seems to largely be to block, or reverse, unfavourable pathways rather than to actively promote favourable ones. The first place at which it may assist is as the Cpn60 protein, with client protein bound, undergoes large movements to a conformation which can bind to Cpn10. As the client protein is bound to several positions on the Cpn60 protein complex, this may stretch the client protein and pull it out of any misfolded conformations that it might have entered. Subsequently, folding in the cavity prevents the client protein from interacting with any other folding proteins, which would be much more likely to occur in the crowded environment of the cytoplasm and which could lead to the formation of aggregates. It is also possible that confinement in the limited volume of the folding cavity may favour folding which minimises the volume of the folding protein.

Although there are some early reports of Cpn60 proteins associating with ribosomes, most evidence suggests that the action of Cpn60 proteins is on fully translated but unfolded or partially folded proteins. Some proteins bind Cpn60 but can also fold in its absence, others require it for folding under stress conditions, and others require it for folding at all times (Kerner et al. 2005). The most recent studies suggest that probably less than 60 proteins are in this last class, but that several of them are essential, which explains the essential nature of Cpn60 (Fujiwara et al. 2010). Numerous studies have attempted to define the rules that determine whether or not a given protein is likely to be a client of Cpn60, but a recent review of these approaches has concluded that to date they are not particularly successful, and that

we cannot yet predict from physico-chemical properties alone whether or not a given protein will be a Cpn60 client *in vivo* (Azia et al. 2012).

Much of the evidence on the function of the Cpn60 proteins as cytoplasmic chaperones is based on experiments done on *E. coli* and on the GroEL protein; as will be seen below, there are important observations from other organisms that show they have additional functions and locations which are of importance in consideration of their roles as moonlighting proteins.

1.4.2 The DnaK/DnaJ Chaperone Machine

The DnaK/DnaJ chaperone pair act upstream of the Cpn60/Cpn10 pair by a very different mechanism. They are non-essential for *E. coli* growth under normal conditions but are required for growth above 42 °C (Bukau and Walker 1998), but nonetheless many studies show they have a central role in protein folding, in particular in recovery from heat shock. Both proteins are abundant in the cell, and the levels of both are increased by the HSR. Like Cpn60 proteins, they are widespread through different domains, although they are absent from many archaea. The DnaK protein is the major bacterial Hsp70 homologue. The DnaJ protein has many homologues, although homology is often limited to a particular domain in the protein, called the J-domain, which is responsible for the interaction with DnaK homologues (Kelley 1998). Many species including *E. coli* contain several homologues of each protein, often with different functions. The discussion below solely concerns the DnaK/DnaJ pair, as these have the most central role in the cell.

Cpn60 proteins act by encapsulating their clients, whereas DnaK and DnaJ act by binding short amino-acid sequences. Analysis of these sequences shows that they are similar to sequences that will be mostly buried in proteins when they become folded (Rüdiger et al. 2001). One major role of the DnaK/DnaJ machine therefore appears to be to protect such sequences from forming incorrect associations with similar sequences on other proteins, which would lead to protein aggregation, until such time as the protein has folded and the sequences are no longer accessible. The ability to bind these sequences also means that the DnaK/J complex is implicated in disassembly of some oligomers, and in resolubilisation of protein aggregates. As with Cpn60 proteins, the DnaK/J complex acts in a cycle of binding and release, mediated by ATP hydrolysis (reviewed in Genevaux et al. 2007). Initially, unfolded proteins with exposed stretches of particular amino-acids bind to DnaJ, which presents them to DnaK which is in its ATP bound state. The interaction of DnaJ and client with DnaK stimulates ATP hydrolysis and leads to closure of the client binding site, protecting these regions on the protein, and to dissociation of the DnaJ protein. A single protein may, in its unfolded state, have several molecules of DnaK-ADP bound in this fashion. The cycle is completed by interaction with GrpE, a nucleotide-exchange factor, which catalyses the replacement of ADP with ATP and hence opening of the binding site and release of the bound protein from DnaK. Many, though not all, DnaK clients are subsequently captured by Cpn60 for further chaperoned protein folding.

A major role of DnaK in non-stressed cells is in transient protection of nascent chains of proteins which are emerging from the ribosome and which hence do not yet have all the information present that is needed for them to fold. The reason that deletion of *dnaK* is not lethal is that DnaK shares this role with another protein, trigger factor (Deuerling et al. 1999). Simultaneous deletion of both genes is lethal at temperatures above 30 °C, though this can be suppressed to an extent by over-expression of GroES and GroEL – demonstrating that chaperones can and do substitute for each other, despite their radically different mechanisms of action, under sufficient duress (Teter et al. 1999; Vorderwülbecke et al. 2005).

The central role of the DnaK/DnaJ machine in the overall protein folding network of the cell has been confirmed by a recent extensive proteomic study of the client proteins of both DnaK and TF (Calloni et al. 2012). This study shows that up to 25 % of cytoplasmic proteins interact with DnaK. Many of these are newly synthesised proteins, but there is evidence (as there is for GroEL) that some proteins repeatedly rebind to DnaK, suggesting that they are relatively unstable and that without regular chaperone maintenance, they would lose their function. DnaK clients are statistically more likely to form aggregation-prone intermediates as they fold, explaining why they need DnaK for efficient folding in the cell. This work also confirmed that many proteins that bind to DnaK go on to interact with GroEL, and reinforced the view that DnaK has a key central role in the chaperone network in the cell, although this role can to some extent be fulfilled by trigger factor if DnaK is absent. This redundancy may be important as DnaK has a wide range of other functions in the cell following stress. Interestingly, many DnaK clients are of relatively low abundance in the cell, which, together with data that shows that the tendency for proteins to aggregate is inversely correlated to their relative abundance (Tartaglia et al. 2007), implies that highly abundant proteins are under selection to evolve to be able to fold without the intercession of molecular chaperones.

The DnaK/DnaJ machine has many roles in survival of stress, often acting with other elements of the HSR in carrying out their function; thus, mutants in *dnaK*, although they are viable under normal conditions of growth, show generally reduced tolerance of stress. In these functions, the role of DnaK/DnaJ often appears to be one of reactivating proteins which have become damaged or unfolded in some way and have become transiently bound to "holders" – chaperones that bind unfolded proteins and prevent their degradation, but which cannot actively refold them. Examples include acting to refold proteins which have become bound to Hsp31 or to the small HSPs IbpA and IbpB after severe heat stress (Mujacic et al. 2004; Mogk et al. 2003); refolding proteins which have become bound to the redox-activated chaperone Hsp33 after oxidative stress (Hoffmann et al. 2004); and acting in concert with the prokaryotic Hsp90 homologue, HtpG, to reactivate proteins that have become aggregated (Genest et al. 2011). Many of these processes appear to be important only after fairly severe stress, which partly explains why neither DnaK, nor the proteins that it operates with, are essential under normal growth conditions. A key role that has been explored in some detail is its ability to refold proteins which have become trapped in protein aggregates, which it does in concert with the

heat shock induced AAA+-protease ClpB (reviewed in Doyle and Wickner 2009). Finally, DnaK and its partners are involved in directing some proteins to proteases, many of which are also HSPS, as is seen in the autoregulation of DnaK by the degradation of σ^{32} by FtsH, discussed above.

1.4.3 Further Examples of Components in the Cytoplasmic Proteostasis Network

In addition to the two major chaperone machines described above at the centre of the proteostasis network in *E. coli*, many other proteins that have a role in protecting, refolding, or degrading other proteins either under normal growth conditions or after exposure to different kinds of stresses. Some of these are discussed below, but the list is far from exhaustive. In particular, proteases are not discussed, but it is important to note that they have an essential role to play in removing damaged proteins from stressed cells (discussed in more detail in Chap. 24). The ways in which the activities of the various different chaperones and proteases are integrated in the overall proteostasis network (reviewed recently in Mogk et al. 2011) is not yet fully understood. However, a mathematical model (EcoFold) of the network (Powers et al. 2012) gives good agreement with experimental data, and it is likely that all the significant components have now been identified.

1.4.3.1 The Small Heat Shock Proteins

Small heat shock proteins are a large and very heterogenous group of proteins which are found in most organisms. In *E. coli*, they are represented by two proteins that are encoded by a single operon with two genes, *ibpA* and *ibpB*. They were first discovered in association with inclusion bodies (hence their name) and are strongly induced by heat shock, particularly at higher temperatures (Allen et al. 1992). However, they are not essential for the cell and their deletion leads to only moderate phenotypes even under heat shock conditions (Thomas and Baneyx 1998). Small heat shock proteins are examples of the class of proteins sometimes referred to generically as "holders". These are proteins whose role is thought to be mainly to protect unfolded proteins by holding them in a conformation where they are not degraded, until they can be refolded by "folders" – the more active, ATP-driven chaperones. Studies using purified proteins show that when both Ibp proteins are present during thermal denaturation of client proteins, those proteins can be more efficiently reactivated by the DnaK chaperone machine interacting with ClpB (Mogk et al. 2003; Matuszewska et al. 2005). *In vivo* data confirms that these proteins assist during extreme heat shock in keeping proteins in a state that can be re-activated (Kuczyńska-Wiśnik et al. 2002).

1.4.3.2 HtpG, the Bacterial Hsp90

Hsp90 proteins are important in eukaryotic cells, where they interact with a wide range of cochaperones and clients (described in Chap. 2) but in *E. coli* and other bacteria, their role is not so central. The sole representative in the *E. coli* genome is called HtpG, and, as is the case with the Ibp proteins described above, this protein is strongly induced upon heat shock but its absence does not lead to a severe phenotype (Bardwell and Craig 1988). It has been shown both *in vivo* and *in vitro* to interact with the DnaK chaperone machinery to assist in the refolding of proteins which have become denatured by heat, though its mechanism is not known (Thomas and Baneyx 1998, 2000; Genest et al. 2011). Its function thus appears to overlap to some extent with that of the small heat shock proteins, an interesting observation given that HtpG is an ATPase and the sHSPs are not.

1.4.4 Chaperones in the Periplasm

The second major semi-aqueous compartment in *E. coli* and other Gram negative bacteria, in addition to the cytoplasm, is the periplasm. This compartment represents between 20 and 40 % of the total cell volume (Stock et al. 1977), and differs in many important ways from the cytoplasm. It is less well separated from the external milieu, is oxidising, contains no ATP, and is an important physical location in its own right, but also for some of the proteins which are *en route* to the outer membrane or outside the cell altogether. Many proteins arrive in the periplasm in an unfolded state, having been kept that way in order to traverse the secretory machinery, and many need to be inserted into the outer membrane. All of these factors mean that it has its own repertoire of chaperones and folding catalysts as described below. These have been recently reviewed in Merdanovic et al. 2011, and are only discussed briefly here.

In the same way as the cell mounts a response to the presence of unfolded proteins in the cytoplasm, a separate response exists that regulates the levels of periplasmic proteins which have a role in protein folding. Indeed, identifying proteins whose levels are increased following the accumulation of unfolded proteins in the periplasm (such as outer membrane protein precursors) is one of the ways that their roles in protein folding were first discovered. Stresses in the periplasm are detected and communicated to the cell in at least two different ways (reviewed by Alba and Gross 2004; Vogt and Raivio 2012). Intriguingly, these systems are also involved in responding to events such as adhesion and so are also likely to play a role in mediating the changes in gene expression that take place during infection.

Many of the proteins with a role in periplasmic protein folding and insertion of outer membrane proteins are involved in covalent modification of the proteins; these are discussed in the next section. One protein which does appear to act as a chaperone by binding and holding proteins and hence reducing the likelihood of their aggregation is called Skp (also known as OmpH). Although this protein can bind to

several outer membrane proteins, deletion of its cognate gene is not lethal and does not cause a reduction in outer membrane protein insertion. However, simultaneous loss of SurA, another periplasmic protein with a protein folding role, is lethal, and proteomics analysis shows that Skp and SurA overlap in their client specificity such that at least one of them has to be present (Denoncin et al. 2012), a situation reminiscent of the trigger factor/DnaK pairing discussed above.

1.5 Covalent Modifiers of Protein Folding

In addition to molecular chaperones, which assist protein folding but do not alter the nature of the covalent bonds in proteins, there exist another set of proteins whose function is needed for efficient protein folding in bacteria. They are genuine enzymes with proteins as their substrates, unlike the chaperones. They consist of enzymes involved in formation and isomerisation of disulphide bonds, and enzymes which can catalyse *cis/trans* isomerisation of the peptide bond at X-Pro, where X is any amino-acid and Pro is proline. Disulphide bond formation and isomerisation take place almost exclusively in the periplasm, except in bacterial strains that have been deliberately engineered to have an oxidising cytoplasm, but proline *cis/trans* isomerisation may occur either in the cytoplasm or the periplasm. Indeed, two of the molecular chaperones discussed above (trigger factor and DnaJ) have this activity, although the extent to which it is important *in vivo* is not clear (reviewed in Wang and Tsou 1998; Hoffmann et al. 2010). The genes for these proteins are generally not essential, but knockout mutants show a variety of different phenotypes including slow growth in some conditions, and some double knock-outs show synthetic lethality.

Catalysis of disulphide bond formation is needed in the periplasm, despite the oxidising nature of that compartment, for efficient folding of proteins that contain disulphide bonds. The *E. coli* periplasm contains a network of proteins which connect the formation of disulphide bonds with electron transport. Disulphide bond formation is catalysed by the DsbA protein, which itself becomes reduced in this process (Bardwell et al. 1991). DsbA is then re-oxidised by the protein DsbB, and DsbB in turn is oxidised directly by interacting with the membrane quinone pool (Kobayashi et al. 1997). Loss of either of these two proteins results in defects such as a loss of motility (due to one of the flagellar proteins being unstable in its reduced form). Another protein, DsbC, can substitute for DsbA to a limited extent but is more active as a disulphide bond isomerase, rearranging disulphide bonds rather than introducing them (Missiakas et al. 1994; Shevchik et al. 1994).

Many genes are annotated as proline cis/trans isomerases in the *E. coli* genome and in the genomes of other bacteria, and the proteins they encode are found both in the cytoplasm and the periplasm. Although none are essential for growth, they have important roles in the cell, most notably in outer membrane biogenesis. Intriguingly, in some cases it has been shown that some aspects of their cellular action is not dependent on their PPIase activity, suggesting them to be proteins with a chaperone

role distinct from their catalytic role. For example, high levels of the periplasmic PPIase FkpA can suppress the formation of periplasmic inclusion bodies even if its PPIase activity is abolished by mutation (Arié et al. 2001).

In summary, there are many components to the bacterial HSR, which together with covalent modifiers of protein folding act together in a network to maintain cytoplasmic and periplasmic proteostasis in both normal and stressed cells, by a combination of protection of unfolded proteins from aggregation, refolding of nascent or pre-existing proteins, and protein degradation. The full workings and inter-relationships of the network remain to be completely described, but the two major chaperone machines of the cell, typified in *E. coli* by the GroEL/GroES and DnaK/DnaJ machines, are crucial to the functioning of the cytoplasmic network. The chaperonins are important because there are several essential proteins which cannot fold in their absence, and the DnaK/DnaJ machine is important in its interacting with numerous other components of the HSR, and other stress regulons, to promoter either protein refolding or protein degradation. A separate network exists in the periplasm, which is essential for outer membrane protein insertion and for the protection of periplasmic proteins from different stresses.

1.6 The Bacterial HSR, Proteostasis, and Infection

Given the significance of the HSR for viability of bacteria under different conditions, and given the obvious fact that bacteria which are causing infections are likely to be under considerable stress from host defences, it is not surprising that evidence has accumulated that shows induction of components of the HSR on infection, and some experiments have demonstrated that key proteins in the HSR are important in aiding bacteria during infection. What was perhaps more surprising was the realisation that stress proteins could and did have multiple roles in infection, which are not always necessarily related to their roles as understood from the *E. coli* paradigm. In this final section, I will review a selection of the evidence that shows that components of the HSR are also important in infection, and then briefly point to some of the evidence that additional roles have evolved for some stress proteins; this volume will of course deal with this topic in detail.

Evidence of induction of expression of chaperones on infection has been reported in numerous different bacteria, using both pre- and post-genomic methods. It was first reported for Salmonella infecting macrophages, where the homologues of GroEL and DnaK are among several proteins which are strongly induced (Buchmeier and Heffron 1990). Intriguingly, a later transcriptomic study of *S. enterica* bv. Typhimurium infecting macrophage showed a *decrease* of *groEL* transcription, although *dnaK* was up-regulated, and the genes for the small heat shock proteins IbpA and IbpB were very strongly up-regulated, showing again the danger of over-generalising from single studies on the HSR (Eriksson et al. 2003). Up-regulation of components of the HSR on infection, typically including Cpn60/10 proteins and DnaK/DnaJ homologues, has since been reported in many other bacteria, including *Staphylococcus aureus* (Qoronfleh et al. 1998), *Listeria monocytogenes* (Gahan

et al. 2001), *Mycobacterium tuberculosis* (Monahan et al. 2001), *Chlamydia trachomatis* (Gérard et al. 2004), *Rickettsia prowazekii* (Gaywee et al. 2002), *Neisseria gonorrhaeae* (Du et al. 2005), and others.

In isolation, up-regulation of gene expression or protein level does not provide conclusive evidence for a role of the proteins concerned. Such evidence relies on studies done on mutant bacteria where expression of the proteins in question is altered in some way, and interpretation of the data from such experiments is complicated by the very pleiotropic nature of mutants in some components of the HSR. Nevertheless, convincing evidence does exist. For example, it has been shown that if the normal heat shock regulation *dnaK* gene of *Brucella suis* is removed, the bacteria can still invade macrophages but cannot multiply in them, and are rapidly lost in a mouse model of infection (Köhler et al. 1996, 2002). Similarly, a DnaK-DnaJ depleted mutant of *S. enterica* bv. Typhimurium cannot survive in macrophages or cause systemic disease in mice (Takaya et al. 2004).

Experiments on the roles of the chaperonin genes in infection are more challenging because of the essential nature of these proteins. However, in many bacteria there are multiple copies of the chaperonin genes (Lund 2009), some of which are non-essential, and the role of one of these has been studied in Mycobacterial infection. It was shown that when the non-essential *cpn60* gene was deleted, *M. tuberculosis* could still grow as normal but it failed to form granulomas in experimental mice and guinea pigs (Hu et al. 2008). This is likely to be due to the fact that chaperonins are potent immunomodulators, a role which is probably independent of their role as chaperones. This unexpected but important aspect of their biology has been reviewed elsewhere (Maguire et al. 2002; Henderson et al. 2010).

1.7 Escaping from the *E. coli* Paradigm

This last observation brings up a central point which is that there is now excellent evidence, in particular from studies on pathogenic bacteria, and their interactions with their hosts, that we must think beyond the *E. coli* paradigm if we are to understand the role or roles of molecular chaperones in infection. Certainly they are important in protection against stress, as is shown in the studies above on their roles in aiding survival in macrophages. But they clearly have additional roles where their chaperone function is probably incidental.

A striking example of this is the finding that for many bacterial species, a Cpn60 protein acts externally to promote adherence to target cells. This has been shown for several very diverse bacterial species to date, including *M. tuberculosis* (where DnaK was also found on the cell surface), *Helicobacter pylori*, *Salmonella enterica* bv. Thyphimurium, *Clostridium difficile*, *Lactobacillus johnsonii*, *Brucella abortus*, *Legionella pneumophila*, and *Haemophilus influenzae* (see Hickey et al. 2009, 2010, and references therein – this is also described by Richard Stokes in Chap. 8). A recent paper even showed that some *E. coli* GroEL was found on the cell surface, and that if this amount was increased, macrophage clearing of *E. coli* cells was enhanced (Zhu et al. 2013).

On the face of it, this is a surprising finding. First, it is not at all clear how Cpn60 proteins get to the outside of the cell; they possess no recognisable signal sequence, and once assembled into large complexes would be expected to be impossible to transport across membranes. Second, Cpn60 proteins require ATP for their normal cellular function, and ATP is not expected to be present in high concentrations outside of the cell. Third, the complete chaperonin cycle also requires Cpn10, but it is hard to see how sufficient levels of Cpn10 could be maintained outside the cell. Cpn60 proteins are notoriously "sticky" and there has always been concern expressed that these results could be artefacts resulting from lysis of some cells followed by binding of liberated Cpn60 to the surface of other cells in the same culture, but careful controls in several of these studies make this interpretation very unlikely. Thus, it seems likely that Cpn60 proteins are genuinely operating to promote some aspect of attachment or adhesion, and they do so not as chaperones in the strict sense. They may act as relatively non-specific "sticky surfaces", but some studies have implicated particular receptors (for example, the LOX-1 receptor) as being involved in recognition of surface- exposed chaperonin proteins. The mechanism by which chaperonins reach the bacterial surface remains unknown, and this is an important area for future study.

1.8 Conclusions

This brief review has shown that bacteria contain highly adapted systems for detecting and surviving the many stresses and shocks to which they may be exposed, and we have a reasonable understanding of some of these. The HSR – more correctly called the unfolded protein response – involves the co-ordinated action of the Cpn60/Cpn10 and DnaK/DnaJ machines, which both also have central roles in normal cellular growth. Together with the other components of the HSR and other proteins which are not part of the heat shock regulon, these two machines assure that proteins are folded to their final native states or, if necessary, degraded. These systems also appear to have key roles to play in bacterial infection, not only by protecting the cells from the stresses that inevitably result, but by providing additional functions which are not necessarily related to their normal chaperone functions. One of these is that Cpn60, normally thought of as an ATP-dependent cytoplasmic chaperone, can also act on the cell surface as an adhesin, facilitating the attachment of bacterial cells to eukaryotic cells. This and other potential roles of these proteins will be discussed in more detail in the current volume.

References

Alba BM, Gross CA (2004) Regulation of the *Escherichia coli* sigma-dependent envelope stress response. Mol Microbiol 52:613–619

Allen SP, Polazzi JO, Gierse JK, Easton AM (1992) Two novel heat shock genes encoding proteins produced in response to heterologous protein expression in *Escherichia coli*. J Bacteriol 174:6938–6947

Arié JP, Sassoon N, Betton JM (2001) Chaperone function of FkpA, a heat shock prolyl isomerase, in the periplasm of *Escherichia coli*. Mol Microbiol 39:199–210
Azia A, Unger R, Horovitz A (2012) What distinguishes GroEL substrates from other *Escherichia coli* proteins? FEBS J 279:543–550
Balch WE, Morimoto RI, Dillin A, Kelly JW (2008) Adapting proteostasis for disease intervention. Science 319:916–919
Bardwell JC, Craig EA (1988) Ancient heat shock gene is dispensable. J Bacteriol 170:2977–2983
Bardwell JC, McGovern K, Beckwith J (1991) Identification of a protein required for disulfide bond formation *in vivo*. Cell 67:581–589
Braig K, Otwinowski Z, Hegde R, Boisvert DC, Joachimiak A, Horwich AL, Sigler PB (1994) The crystal structure of the bacterial chaperonin GroEL at 2.8 Å. Nature 371:578–586
Buchmeier NA, Heffron F (1990) Induction of Salmonella stress proteins upon infection of macrophages. Science 248:730–732
Bukau B, Walker GC (1998) Cellular defects caused by deletion of the Escherichia coli dnaK gene indicate roles for heat shock protein in normal metabolism. J Bacteriol 171:2337–2346
Calloni G, Chen T, Schermann SM, Chang HC, Genevaux P, Agostini F, Tartaglia GG, Hayer-Hartl M, Hartl FU (2012) DnaK functions as a central hub in the *E. coli* chaperone network. Cell Rep 1:251–264
Chaudhuri S, Jana B, Basu T (2006) Why does ethanol induce cellular heat-shock response? Cell Biol Toxicol 22:29–37
Clark MS, Peck LS (2009) HSP70 heat shock proteins and environmental stress in Antarctic marine organisms: a mini-review. Mar Genomics 2:11–18
Denoncin K, Schwalm J, Vertommen D, Silhavy TJ, Collet JF (2012) Dissecting the *Escherichia coli* periplasmic chaperone network using differential proteomics. Proteomics 12:1391–1401
Deuerling E, Schulze-Specking A, Tomoyasu T, Mogk A, Bukau B (1999) Trigger factor and DnaK cooperate in folding of newly synthesized proteins. Nature 400:693–696
Doyle SM, Wickner S (2009) Hsp104 and ClpB: protein disaggregating machines. Trends Biochem Sci 34:40–48
Du Y, Lenz J, Arvidson CG (2005) Global gene expression and the role of sigma factors in *Neisseria gonorrhoeae* in interactions with epithelial cells. Infect Immun 73:4834–4845
El-Samad H, Kurata H, Doyle JC, Gross CA, Khammash M (2005) Surviving heat shock: control strategies for robustness and performance. Proc Natl Acad Sci U S A 102:2736–2741
Eriksson S, Lucchini S, Thompson A, Rhen M, Hinton JC (2003) Unravelling the biology of macrophage infection by gene expression profiling of intracellular *Salmonella enterica*. Mol Microbiol 47:103–118
Fayet O, Ziegelhoffer T, Georgopoulos C (1989) The groES and groEL heat shock gene products of *Escherichia coli* are essential for bacterial growth at all temperatures. J Bacteriol 171:1379–1385
Foster JW (2004) *Escherichia coli* acid resistance: tales of an amateur acidophile. Nat Rev Microbiol 2:898–907
Fujiwara K, Ishihama Y, Nakahigashi K, Soga T, Taguchi H (2010) A systematic survey of *in vivo* obligate chaperonin-dependent substrates. EMBO J 29:1552–1564
Gahan CG, O'Mahony J, Hill C (2001) Characterization of the *groESL* operon in *Listeria monocytogenes*: utilization of two reporter systems (*gfp* and *hly*) for evaluating *in vivo* expression. Infect Immun 69:3924–3932
Gaywee J, Radulovic S, Higgins JA, Azad AF (2002) Transcriptional analysis of *Rickettsia prowazekii* invasion gene homolog (*invA*) during host cell infection. Infect Immun 70:6346–6354
Genest O, Hoskins JR, Camberg JL, Doyle SM, Wickner S (2011) Heat shock protein 90 from *Escherichia coli* collaborates with the DnaK chaperone system in client protein remodeling. Proc Natl Acad Sci U S A 108:8206–8211
Genevaux P, Georgopoulos C, Kelley WL (2007) The Hsp70 chaperone machines of *Escherichia coli*: a paradigm for the repartition of chaperone functions. Mol Microbiol 66:840–857
Gérard HC, Whittum-Hudson JA, Schumacher HR, Hudson AP (2004) Differential expression of three Chlamydia trachomatis hsp60-encoding genes in active vs. persistent infections. Microb Pathog 36:35–39

Goff SA, Goldberg AL (1985) Production of abnormal proteins in *E. coli* stimulates transcription of *lon* and other heat shock genes. Cell 41:587–595

Gragerov AI, Martin ES, Krupenko MA, Kashlev MV, Nikiforov VG (1991) Protein aggregation and inclusion body formation in *Escherichia coli rpoH* mutant defective in heat shock protein induction. FEBS Lett 291:222–224

Gragerov A, Nudler E, Komissarova N, Gaitanaris GA, Gottesman ME, Nikiforov V (1992) Cooperation of GroEL/GroES and DnaK/DnaJ heat shock proteins in preventing protein misfolding in *Escherichia coli*. Proc Natl Acad Sci U S A 89:10341–10344

Guisbert E, Yura T, Rhodius VA, Gross CA (2008) Convergence of molecular, modelling, and systems approaches for an understanding of the *Escherichia coli* heat shock response. Microbiol Mol Biol Rev 72:45–54

Hemmingsen SM, Woolford C, van der Vies SM, Tilly K, Dennis DT, Georgopoulos CP, Hendrix RW, Ellis RJ (1988) Homologous plant and bacterial proteins chaperone oligomeric protein assembly. Nature 333:330–334

Henderson B, Lund PA, Coates AR (2010) Multiple moonlighting functions of mycobacterial molecular chaperones. Tuberculosis 90:119–124

Hickey TB, Thorson LM, Speert DP, Daffé M, Stokes RW (2009) *Mycobacterium tuberculosis* Cpn60.2 and DnaK are located on the bacterial surface, where Cpn60.2 facilitates efficient bacterial association with macrophages. Infect Immun 77:3389–3401

Hickey TB, Ziltener HJ, Speert DP, Stokes RW (2010) *Mycobacterium tuberculosis* employs Cpn60.2 as an adhesin that binds CD43 on the macrophage surface. Cell Microbiol 12:1634–1647

Hoffmann A, Bukau B, Kramer G (2010) Structure and function of the molecular chaperone Trigger Factor. Biochim Biophys Acta 1803:650–661

Hoffmann JH, Linke K, Graf PC, Lilie H, Jakob U (2004) Identification of a redox-regulated chaperone network. EMBO J 23:160–168

Horwich AL, Fenton WA (2009) Chaperonin-mediated protein folding: using a central cavity to kinetically assist polypeptide chain folding. Q Rev Biophys 42:83–116

Horwich AL, Fenton WA, Chapman E, Farr GW (2007) Two families of chaperonin: physiology and mechanism. Annu Rev Cell Dev Biol 23:115–145

Hu Y, Henderson B, Lund PA, Tormay P, Ahmed MT, Gurcha SS, Besra GS, Coates AR (2008) A *Mycobacterium tuberculosis* mutant lacking the groEL homologue Cpn60.1 is viable but fails to induce an inflammatory response in animal models of infection. Infect Immun 76:1535–1546

Inoue M, Mitarai N, Trusina A (2012) Circuit architecture explains functional similarity of bacterial heat shock responses. Phys Biol 9:066003

Ishihama Y, Schmidt T, Rappsilber J, Mann M, Hartl FU, Kerner MJ, Frishman D (2008) Protein abundance profiling of the *Escherichia coli* cytosol. BMC Genomics 9:1027–1039

Kapatai G, Large A, Benesch JL, Robinson CV, Carrascosa JL, Valpuesta JM, Gowrinathan P, Lund PA (2006) All three chaperonin genes in the archaeon *Haloferax volcanii* are individually dispensable. Mol Microbiol 61:1583–1597

Kelley WL (1998) The J-domain family and the recruitment of chaperone power. Trends Biochem Sci 23:222–227

Kerner MJ, Naylor DJ, Ishihama Y, Maier T, Chang HC, Stines AP, Georgopoulos C, Frishman D, Hayer-Hartl M, Mann M, Hartl FU (2005) Proteome-wide analysis of chaperonin-dependent protein folding in *Escherichia coli*. Cell 122:209–220

Kobayashi T, Kishigami S, Sone M, Inokuchi H, Mogi T, Ito K (1997) Respiratory chain is required to maintain oxidized states of the DsbA-DsbB disulfide bond formation system in aerobically growing *Escherichia coli* cells. Proc Natl Acad Sci U S A 94:11857–11862

Köhler S, Teyssier J, Cloeckaert A, Rouot B, Liautard JP (1996) Participation of the molecular chaperone DnaK in intracellular growth of *Brucella suis* within U937-derived phagocytes. Mol Microbiol 20:701–712

Köhler S, Ekaza E, Paquet JY, Walravens K, Teyssier J, Godfroid J, Liautard JP (2002) Induction of *dnaK* through its native heat shock promoter is necessary for intramacrophagic replication of *Brucella suis*. Infect Immun 70:1631–1634

Kuczyńska-Wiśnik D, Kedzierska S, Matuszewska E, Lund P, Taylor A, Lipińska B, Laskowska E (2002) The *Escherichia coli* small heat-shock proteins IbpA and IbpB prevent the aggregation of endogenous proteins denatured in vivo during extreme heat shock. Microbiology 148:1757–1765

Kusukawa N, Yura T (1988) Heat shock protein GroE of *Escherichia coli*: key protective roles against thermal stress. Genes Dev 2:874–882

Lund PA (2009) Multiple chaperonins in bacteria–why so many? FEMS Microbiol Rev 33:785–800

Maguire M, Coates AR, Henderson B (2002) Chaperonin 60 unfolds its secrets of cellular communication. Cell Stress Chaperones 7:317–329

Matuszewska M, Kuczynska-Wisnik D, Laskowska E, Liberek K (2005) The small heat shock protein IbpA of *Escherichia coli* cooperates with IbpB in stabilization of thermally aggregated proteins in a disaggregation competent state. J Biol Chem 280:12292–12298

Merdanovic M, Clausen T, Kaiser M, Huber R, Ehrmann M (2011) Protein quality control in the bacterial periplasm. Annu Rev Microbiol 65:149–168

Mira A, Ochman H, Moran NA (2001) Deletional bias and the evolution of bacterial genomes. Trends Genet 17:589–596

Missiakas D, Georgopoulos C, Raina S (1994) The *Escherichia coli* dsbC (xprA) gene encodes a periplasmic protein involved in disulfide bond formation. EMBO J 13:2013–2020

Mogk A, Deuerling E, Vorderwülbecke S, Vierling E, Bukau B (2003) Small heat shock proteins, ClpB and the DnaK system form a functional triage in reversing protein aggregation. Mol Microbiol 50:585–595

Mogk A, Huber D, Bukau B (2011) Integrating protein homeostasis strategies in prokaryotes. *Cold Spring Harb Perspect Biol* 3: pii: a004366. doi:10.1101/cshperspect.a004366

Monahan IM, Betts J, Banerjee DK, Butcher PD (2001) Differential expression of mycobacterial proteins following phagocytosis by macrophages. Microbiology 147:459–471

Mujacic M, Bader MW, Baneyx F (2004) *Escherichia coli* Hsp31 functions as a holding chaperone that cooperates with the DnaK-DnaJ-GrpE system in the management of protein misfolding under severe stress conditions. Mol Microbiol 51:849–859

Narberhaus F (1999) Negative regulation of bacterial heat shock genes. Mol Microbiol 31:1–8

Neidhardt FC, VanBogelen RA, Vaughn V (1984) The genetics and regulation of heat-shock proteins. Annu Rev Genet 18:295–329

Nielsen KL, McLennan N, Masters M, Cowan NJ (1999) A single-ring mitochondrial chaperonin (Hsp60-Hsp10) can substitute for GroEL-GroES *in vivo*. J Bacteriol 181:5871–5875

Nonaka G, Blankschien M, Herman C, Gross CA, Rhodius VA (2006) Regulon and promoter analysis of the *E. coli* heat-shock factor, sigma32, reveals a multifaceted cellular response to heat stress. Genes Dev 20:1776–1789

Parsell DA, Sauer RT (1989) Induction of a heat shock-like response by unfolded protein in *Escherichia coli*: dependence on protein level not protein degradation. Genes Dev 3:1226–1232

Powers ET, Powers DL, Gierasch LM (2012) FoldEco: a model for proteostasis in *E. coli*. Cell Rep 1:265–276

Qoronfleh MW, Bortner CA, Schwartzberg P, Wilkinson BJ (1998) Enhanced levels of *Staphylococcus aureus* stress protein GroEL and DnaK homologs early in infection of human epithelial cells. Infect Immun 66:3024–3027

Richmond CS, Glasner JD, Mau R, Jin H, Blattner FR (1999) Genome-wide expression profiling in *Escherichia coli* K-12. Nucleic Acids Res 27:3821–3835

Ritossa F (1962) A new puffing pattern induced by temperature shock and DNP in Drosophila. Experientia 18:571–573

Rüdiger S, Schneider-Mergener J, Bukau B (2001) Its substrate specificity characterizes the DnaJ co-chaperone as a scanning factor for the DnaK chaperone. EMBO J 20:1042–1050

Shevchik VE, Condemine G, Robert-Baudouy J (1994) Characterization of DsbC, a periplasmic protein of *Erwinia chrysanthemi* and *Escherichia coli* with disulfide isomerase activity. EMBO J 13:2007–20012

Stock JB, Rauch B, Roseman S (1977) Periplasmic space in *Salmonella typhimurium* and *Escherichia coli*. J Biol Chem 252:7850–7861

Stoldt V, Rademacher F, Kehren V, Ernst JF, Pearce DA, Sherman F (1996) The Cct eukaryotic chaperonin subunits of *Saccharomyces cerevisiae* and other yeasts. Yeast 12:523–529

Takaya A, Tomoyasu T, Matsui H, Yamamoto T (2004) The DnaK/DnaJ chaperone machinery of *Salmonella enterica* serovar Typhimurium is essential for invasion of epithelial cells and survival within macrophages, leading to systemic infection. Infect Immun 72:1364–1373

Tartaglia GG, Pechmann S, Dobson CM, Vendruscolo M (2007) Life on the edge: a link between gene expression levels and aggregation rates of human proteins. Trends Biochem Sci 32:204–206

Teter SA, Houry WA, Ang D, Tradler T, Rockabrand D, Fischer G, Blum P, Georgopoulos C, Hartl FU (1999) Polypeptide flux through bacterial Hsp70: DnaK cooperates with trigger factor in chaperoning nascent chains. Cell 97:755–765

Thomas JG, Baneyx F (1998) Roles of the *Escherichia coli* small heat shock proteins IbpA and IbpB in thermal stress management: comparison with ClpA, ClpB, and HtpG *in vivo*. J Bacteriol 180:5165–5172

Thomas JG, Baneyx F (2000) ClpB and HtpG facilitate *de novo* protein folding in stressed *Escherichia coli* cells. Mol Microbiol 36:1360–1370

Trent JD, Osipiuk J, Pinkau T (1990) Acquired thermotolerance and heat shock in the extremely thermophilic archaebacterium *Sulfolobus sp.* strain B12. J Bacteriol 172:1478–1484

Vogt SL, Raivio TL (2012) Just scratching the surface: an expanding view of the Cpx envelope stress response. FEMS Microbiol Lett 326:2–11

Vorderwülbecke S, Kramer G, Merz F, Kurz TA, Rauch T, Zachmann-Brand B, Bukau B, Deuerling E (2005) Low temperature of GroEL/ES overproduction permits growth of *Escherichia coli* cells lacking trigger factor DnaK. FEBS Lett 579:181–187

Wang CC, Tsou CL (1998) Enzymes as chaperones and chaperones as enzymes. FEBS Lett 425:382–384

Wild J, Walter WA, Gross CA, Altman E (1993) Accumulation of secretory protein precursors in *Escherichia coli* induces the heat shock response. J Bacteriol 175:3992–3997

Yébenes H, Mesa P, Muñoz IG, Montoya G, Valpuesta JM (2011) Chaperonins: two rings for folding. Trends Biochem Sci 36:424–432

Zhou YN, Kusukawa N, Erickson JW, Gross CA, Yura T (1988) Isolation and characterization of *Escherichia coli* mutants that lack the heat shock sigma factor sigma 32. J Bacteriol 170:3640–3649

Zhu H, Lee C, Zhang D, Wu W, Wang L, Fang X, Xu X, Song D, Xie J, Ren S, Gu J (2013) Surface-associated GroEL facilitates the adhesion of *Escherichia coli* to macrophages through lectin-like oxidized low-density lipoprotein receptor-1. Microbes Infect 15:72–80