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Circuit architecture explains functional similarity of bacterial heat shock responses

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Abstract
Heat shock response is a stress response to temperature changes and a consecutive increase in amounts of unfolded proteins. To restore homeostasis, cells upregulate chaperones facilitating protein folding by means of transcription factors (TFs). We here investigate two heat shock systems: one characteristic to gram negative bacteria, mediated by transcriptional activator σ^{32} in *E. coli*, and another characteristic to gram positive bacteria, mediated by transcriptional repressor HrcA in *L. lactis*. We construct simple mathematical models of the two systems focusing on the negative feedbacks, where free chaperones suppress σ^{32} activation in the former, while they activate HrcA repression in the latter. We demonstrate that both systems, in spite of the difference at the TF regulation level, are capable of showing very similar heat shock dynamics. We find that differences in regulation impose distinct constraints on chaperone–TF binding affinities: the binding constant of free σ^{32} to chaperone DnaK, known to be in 100 nM range, set the lower limit of amount of free chaperone that the system can sense the change at the heat shock, while the binding affinity of HrcA to chaperone GroE set the upper limit and have to be rather large extending into the micromolar range.

(Some figures may appear in colour only in the online journal)

Introduction

Cellular homeostasis is essential for proper protein folding and function. The perturbations to homeostasis, e.g. due to change in temperature or osmotic pressure, result in protein unfolding or/and misfolding. Heat shock, i.e. a sudden increase of temperature, causes such protein unfolding and misfolding and can result in cell death. To counteract the heat shock, cells upregulate the production of chaperones and proteases—enzymes that help folding the unfolded proteins and degrade terminally misfolded proteins, respectively. The heat shock response is one of the stress responses characteristic to nearly all living organisms. Interestingly, the protein sequence of most chaperones and proteases is well conserved from bacteria to humans [1]. It is, however, unclear if the features of heat shock response are also preserved at the level of the architecture of regulatory circuits governing heat shock response.

In this paper, we attempt to answer this question and derive useful insights by comparing the heat shock in *E. coli* and *L. lactis*. These organisms utilize two different mechanisms: a system with σ^{32} and DnaK in *E. coli* and a system with HrcA and GroE in *L. lactis*. Both mechanisms are widely observed in microorganisms. A transcriptional activator RpoH, σ^{32} homolog, is found in the alpha-, beta-, and gamma-proteobacteria, while a transcriptional inhibitor, HrcA, is widely distributed in eubacteria, but not in the gamma-proteobacteria. Interestingly, there also exist bacteria which have both systems and, furthermore, a regulatory loop between σ^{32} and HrcA is predicted in some beta-proteobacteria [2].

Heat shock responses have been extensively studied both experimentally and theoretically. In experiments, protein sequences and their regulatory mechanisms are revealed in the both systems (figure 1) [3]. While the σ^{32} system is modeled quite a large extent [4, 5], to our knowledge there is no

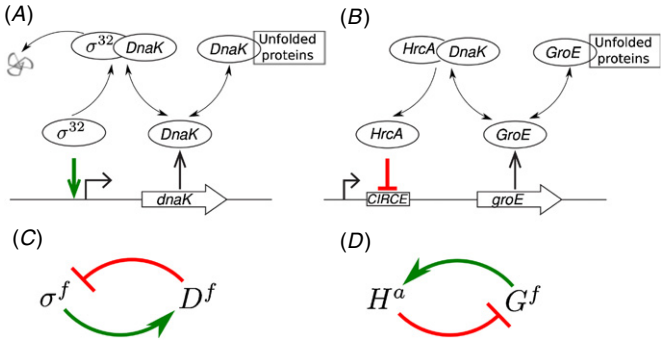

Figure 1. Comparison of the reaction mechanisms between ((*A*) and (*C*)) σ^{32}–DnaK system and ((*B*) and (*D*)) HrcA–GroE system. Views for gene regulations and protein reactions ((*A*) and (*B*)) and outline illustrations for distinct reactions ((*C*) and (*D*)).

modeling work on the HrcA system. Our aim in this study is to construct a simple model based on known experimental data for each system and theoretically investigate similarities and differences in the regulatory features and the dynamical responses mediated by σ^{32} and HrcA.

One of the striking similarities emerges at the level of the dynamics of the transcription regulators: σ^{32} and HrcA. Both systems respond with a sharp peak in the rate of production of new chaperones: upon a temperature shift, a fast increase up to 4–5 fold within 5–10 min (corresponding to about 0.1 generation time [5]) is followed by a rapid decline to a new steady state that is about 1.5 fold of the one at the starting temperature in both σ^{32} and HrcA systems [5, 6]. This similarity is particularly interesting as the mechanisms of transcriptional regulation are very different: while σ^{32} is an activator, HrcA is a repressor (see figure 1).

Model

In this section, we explain how we construct our models based on existing experimental observations.

σ^{32}–DnaK system. Our model with σ^{32} and DnaK is in large part similar to that outlined in [4] with the exception of the differences outlined below. The main players in the model are: σ^{32} (σ; transcription factor), DnaK (D; chaperone) and unfolded proteins (U). (In the following, we will denote total concentration of protein X as $[X^t]$ and the concentration of free protein as $[X^f]$.)

σ^{32} is unstable and is present only in small amounts (a few hundreds proteins per cell at most) [7]. Under steady state conditions it is sequestered by chaperones, such that the most σ^{32} exists as a complex ($[\sigma^t] \sim [\sigma D]$). For simplicity, we assume it is produced at a constant rate independent of temperature. In addition, for a simpler comparison with the HrcA system, we do not include the stabilization of σ^{32} (half-life changes from 1 to 8 min) during the heat shock. These two are the main differences from the model in [4].

When not bound to DnaK, σ^{32} forms a complex with RNA polymerase (RNAp) and targets RNAp for the transcription of heat shock proteins, including DnaK. For simplicity, we will refer to this [RNA$p : \sigma^{32}$] complex as 'free' σ^{32}, $[\sigma^f]$ (i.e. not bound to DnaK).

Being a chaperone, DnaK facilitates proteins folding and thus forms transient complexes with unfolded proteins. A temperature shift destabilizes the folding of existing folded proteins and also hinders the folding of *de novo* synthesized proteins; thus sequestering all the existing chaperones and creating the demand for additional chaperones. The demand for additional chaperones is sensed and mediated by σ^{32}. As long as there are enough unfolded proteins to keep chaperones sequestered away from σ^{32}, it will facilitate the transcription of heat shock proteins. Namely the regulatory network has a negative feedback loop (see figures 1(*A*) and (*C*)), i.e. σ^{32} activates DnaK by transcriptional activation ('slow' reaction), while DnaK inhibits σ^{32} by complex formation ('fast' reaction).

We assume that the reactions $\sigma^f + D^f \rightleftharpoons \sigma D$ and $U^f + D^f \rightleftharpoons UD$ are in equilibrium as the kinetics of complex formation and dissociation between σ^{32} and DnaK and between unfolded proteins and DnaK are much faster than transcription and translation. Based on these observations and assumptions, we describe the system's dynamics with the following equations:

$$[\dot{\sigma^t}] = \eta - \gamma_s[\sigma^t] - \gamma_c[\sigma D], \tag{1}$$

$$[\dot{D^t}] = \alpha_d \frac{[\sigma^f]/K_\sigma}{1 + [\sigma^f]/K_\sigma} - \gamma_s[D^t], \tag{2}$$

$$[\dot{U^t}] = F(T) - \gamma_{us}[UD], \tag{3}$$

$$[\sigma D] = \frac{[\sigma^f][D^f]}{K_j}, \tag{4}$$

$$[UD] = \frac{[U^f][D^f]}{K_k}, \tag{5}$$

with the conditions of mass conservation:

$$[\sigma^t] = [\sigma^f] + [\sigma D], \tag{6}$$

$$[D^t] = [D^f] + [\sigma D] + [UD], \tag{7}$$

$$[U^t] = [U^f] + [UD]. \tag{8}$$

The rate of change in σ^{32} (equation (1)) is given by the constitutive transcription rate, η, dilution due to cell division with the cell doubling, $1/\gamma_s$, and degradation with fast rate, γ_c. Since σ^{32} is degraded mainly through chaperone-dependent degradation by FisH [8], the fast degradation term depends on the complex $[\sigma D]$. The production of chaperone DnaK (first term in equation (2)) is transcriptionally activated by free σ^{32}, $[\sigma^f]$, and parameterized by the maximal production rate α_d. For $[\sigma^f]$ dependence, we adopt the Michaelis–Menten form with a dissociation constant K_σ.

Chaperones are typically stable proteins with the half-life comparable to cell doubling time $1/\gamma_s$, which is included in the second term of equation (2). The first term in equation (3), $F(T)$, represents that the production of unfolded protein is an

increasing function of the temperature, T. Unfolded proteins can re-fold correctly with the help of chaperones and are thus removed at a rate proportional to $[UD]$ with the rate γ_{us} (the second term in equation (3)). Equations (4) and (5) represents complex formations in equilibrium, with K_j and K_k being the dissociation constants between free σ^{32} and free DnaK and between free unfolded protein and free DnaK, respectively[3].

We fix some of the parameter values according to the experimental observations as follows. We set γ_c to a unit time ($= 1$), which shows a fast degradation of a complex σD as most σ^{32} exist as the complex in the steady state. The timescale of the fast degradation, $1/\gamma_c$, is assumed to be around 1 min [7][4]. $1/\gamma_s$ is a timescale for the slow degradation of σ^t and D^t and it is set to the inverse number of cell division time (∼30 time units) [9]. As already mentioned, σ^{32} is present only in small amounts [7]. We here assume $[\sigma^t] \sim 200$ nM and expect the model to work as long as it is within a few hundreds nM range. We estimate $\eta \sim 200$ nM min^{-1} according to this number. The dissociation constant between chaperones and σ^{32} ranges 5 μM to 19 nM between correspondingly DnaK/DnaJ and σ^{32} [8]. We have set the constant to be $K_j = 100$ nM, following the choice in [4].

The rest of parameters, $\alpha_d, K_\sigma, K_k, \gamma_{us}$ and $F(T)$, have been chosen so that the model reproduces experimental observations, i.e. (i) $[D^t] \sim 20\,000$ nM [10, 11]. (ii) DnaK production rate changes 4–6 times for a peak and its new steady state after heat shock becomes 1.5 times of the before heat shock [5]. (iii) The peak time is less than 5 time units[5] and the peak shape of the DnaK production rate is symmetric [7, 5, 6]. (iv) The steady state amount of free unfolded proteins should be kept small both before and after heat shock. This is affected by K_k, γ_{us} and $F(T)$, which are the parameters related to unfolded proteins. In this paper, we fix $K_k = 1$ nM so that U_f is in nanomolar range in the steady state, and fit the other two parameters. We tested higher values of K_k (up to $K_k = 1,000$ nM, which would bring U_f to micromolar range) and they all give a proper response as long as $F(T)$ and γ_{us} adjusted accordingly to account for the timing of the peak.

HrcA–GroE system. Next, we construct a model for HrcA and GroE system, where GroE (G) is a chaperone and HrcA (H) transcriptionally represses GroE. We adopt the reaction mechanism shown in figure 2 in [12]; HrcA repressor is released from the ribosomes as an inactive protein (H^i), which cannot bind to the operator, and it has to interact with the GroE chaperonin system to become active (H^a). The inactive HrcA (H^i) interacts with chaperone GroE (HG) and the active HrcA is released. The active HrcA (H^a) is able to bind to the operator and transcriptionally inhibits the production of GroE, while the active HrcA becomes inactive again at a constant rate, i.e. upon dissociation from its binding site, HrcA is in its inactive form again [13]. In this model, we assume that the total amount of HrcA ($[H^t] = [H^a] + [H^i] + [HG]$) is a constant for simplicity. As in case with DnaK, GroE chaperone makes a complex with an unfolded protein and helps it to refold correctly. Similar to the σ^{32} system, we assume that the two reactions ($H^i + G^f \rightleftharpoons HG$ and $U^f + G^f \rightleftharpoons UG$) are fast compared with other reactions and always in the equilibrium states.

This system also has a negative feedback loop between transcription factor HrcA and chaperone GroE. However, the regulation is opposite; the active HrcA inhibits GroE with a slow reaction (transcriptional inhibition) and GroE activates the inactive HrcA with a fast reaction (enzymatic modification). From the points described, we obtain the following reaction equations:

$$[\dot{H^a}] = \beta_h[HG] - \gamma_c[H^a], \tag{9}$$

$$[\dot{G^t}] = \beta_g \frac{1}{1 + [H^a]/K_h} - \gamma_s[G^t], \tag{10}$$

$$[\dot{U^t}] = F(T) - \gamma_{uh}[UG], \tag{11}$$

$$[HG] = \frac{[H^i][G^f]}{K_l}, \tag{12}$$

$$[UG] = \frac{[U^f][G^f]}{K_m}, \tag{13}$$

with the conditions of mass conservation:

$$[H^t] = [H^a] + [H^i] + [HG], \tag{14}$$

$$[G^t] = [G^f] + [HG] + [UG], \tag{15}$$

$$[U^t] = [U^f] + [UG]. \tag{16}$$

Equation (9) represents the time evolution of $[H^a]$ with constant total amount of HrcA, where the production rate of $[H^a]$ is given by activation from H^i through forming a complex $[HG]$ with the rate β_h, and inactivation happens with a fast rate γ_c. The time evolutions of chaperone $[G^t]$ (equation (10)) and unfolded protein $[U^t]$ (equation (11)) are similar to equations (2) and (3), except that $[G^t]$ is transcriptionally inhibited by $[H^a]$ in equation (10). Here, β_g is the maximum production rate of GroE, K_h is the dissociation constant of active HrcA to GroE promoter and γ_{uh} is the rate of removal of unfolded proteins by GroE. Equations (12) and (13) represent complex formations in equilibrium, with K_l and K_m being the dissociation constants between inactive HrcA and free GroE and between free unfolded protein and free GroE, respectively.

Most of the parameters were not experimentally measured for the HrcA–GroE system. However, for a fair comparison between the two systems, we, whenever possible, use same parameter values as in the σ^{32}–DnaK system (see table 1 for correspondence relation). Thus, we assume similar concentrations for the transcription factor $[H^t] = 200$ nM ($\sim [\sigma^t]$) and chaperones $[G^t] \sim 20\,000$ nM ($\sim [D^t]$). Although these numbers have not been validated

[3] In the numerical simulation of the model, the complex formation was solved by using ordinary differential equations with much faster timescales than equations (1)–(3) for the simplicity of calculation.

[4] From equations (1) and (4), the degradation term is given as $\gamma_c[\sigma_t]/(1 + K_j/[D_f])$. Noting $K_j/[D_f] \sim 1/10$ (see table 1 and figure 4), $1/\gamma_c$ approximately gives the lifetime of σ^{32}.

[5] In the original experiments by Arnvig *et al*, the response to temperature shift from 30 to 37°C was peaking at about 0.1 generations, corresponding to 3–4 min. To address other temperature shifts, the time in our simulations can be rescaled in terms of cell generations such that the key criteria and the main results would still hold.

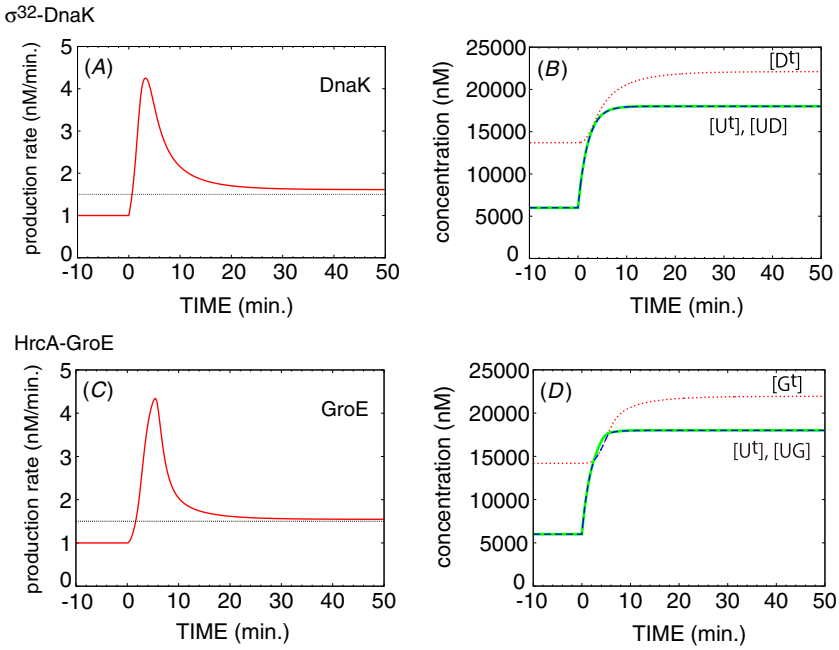

Figure 2. Heat shock response in the σ^{32}–DnaK system ((*A*) and (*B*)) and the HrcA–GroE system ((*C*) and (*D*)). Heat shock is induced at $t = 0$. ((*A*) and (*C*)) shows the time evolution of the chaperone production rate normalized by the pre-stimulus level. ((*B*) and (*D*)) shows the time evolution of the density of total chaperones (dotted lines), total unfolded-protein (bold lines), and complex formed by chaperone and unfolded proteins (broken lines). Parameters used in simulations are shown in table 1.

Table 1. Parameter values used in the model. Parameters marked with $*$ are chosen to reproduce rapid transient response measured in [5, 6]. The rest of the parameters are based on experimental data (references shown in the table: see the text for details).

σ^{32}–DnaK system				HrcA–GroE system			
η		200	[7]	$[H^t]$		200	
γ_c		1	[7]			1	
γ_s		0.03	[9]			0.03	
K_k	$*$	1		K_m	$*$	1	
K_j		100	[8]	K_l	$*$	100 000	
α_d	$*$	2000		β_g	$*$	2000	
K_σ	$*$	10		K_h	$*$	10	
γ_{us}	$*$	0.5		γ_{uh}	$*$	0.5	
				β_h	$*$	3	
$F(T)$	$*$	$3000 \rightarrow 9000$			$*$	$3000 \rightarrow 9000$	

experimentally, they match the typical concentrations of transcription factors (of order 100 nM) and chaperones (of order 10 μM).

The fastest doubling time in *L. lactis* is also about 30 min [14]; thus, we use the same doubling time as in *E. coli* with a corresponding doubling time $\gamma_s = 0.03$. Unlike σ^{32}, HrcA is a stable protein (in *B. subtilis*), with the half-life more than 60 minutes [15]. However, it has been suggested that HrcA is present in two conformations, one is active and another is inactive, and the equilibrium between these two states is modulated by GroEL, which shift the equilibrium toward the active state [13]. Thus γ_c, which we set to be 1, is representing the rate of conversion from the active to inactive state, rather than protein half-life as is the case in σ^{32}.

The time of the peak, fold induction at the peak and fold change of the new steady state are overall similar in the activity of the CIRCA operon [6] (corresponding to GroEL production rate) and σ^{32} governed chaperone (DnaK) production. This allows us to use the same criteria (i)–(iv) to set K_m, β_g, K_h, γ_{uh}, β_h, $F(T)$ and K_l. We study the response of this model assuming the same conditions as for the σ^{32} system (figures 2(*C*) and (*D*)). (In the HrcA model, we assumed $[H^t]$ is constant for simplicity. However, the model also works even if we discard this postulation and include that HrcA inhibits its own transcription [12], i.e. the time evolution of $[H^t]$ will be described as $[\dot{H}^t] = \mu/(1 + [H^a]/K_t) - \gamma_s[H^t]$.)

Results

Model predicts much weaker binding affinity between HrcA and chaperones

In figure 2, we show that both σ^{32}–DnaK and HrcA–GroE systems are able to reproduce experimental observations. Production of chaperones shows a characteristic sharp peak with a fast increase up to 4–6 fold within 5 min and a following decline to a new steady state that is about 1.5 fold of the pre-stimulus one. When choosing unknown parameters, our initial strategy was to use the same values for corresponding parameters in each of the systems (see table 1). Remarkably, this was possible for all but one parameter: the binding affinity of TF to chaperones, K_j and K_l. It appears that while σ^{32} binds tightly to the chaperones ($K_j = 100$ nM), it is essential that HrcA is bound only weakly with a micromolar binding constant ($K_l = 100$ μM).

We next study a response upon an inverse heat shock, i.e. a response when temperature is suddenly decreased. Inverse heat shock response has been studied experimentally in *E. coli* [5, 16] (to our knowledge no data exist for *L. lactis*) and is

characterized by a rapid decrease in chaperone production with a consequent slow increase to a new steady state that is lower than before temperature decrease.

In figure 3, we show the chaperone production rate upon an inverse heat shock. The response is simulated using the parameter values in table 1 except for $F(T)$ which is reversed (suddenly decreased at $t = 0.$). Both models for σ^{32}–DnaK and HrcA–GroE systems showed very similar responses, which fit well with experimental results; the chaperone production rate shows a rapid transient decrease and recovers slower compared with a direct heat shock response. The fact that the model works without specific tuning of parameters to the inverse heat shock supports that our simple models hit the essential points of the actual reaction mechanisms.

Why is the recovery to the new steady state slower in the inverse heat shock in both systems? The explanation naturally emerges from our model: as chaperones are stable proteins, the only way to recover to a new steady state upon decrease in unfolded proteins is by dilution due to cell division. Thus, this slow timescale for the recovery is given by $1/\gamma_s$ or the timescale of cell division in both systems. In the case of direct heat shock, the time to recover to a new steady state (right after the peak, once there are enough chaperones produced) is governed by the turnover time for active TFs, γ_c, which is much faster than the rate governed by cell doubling time γ_s.

The difference in negative feedback architectures requires different constraints on TF–chaperone binding affinities

The reaction mechanisms of the two systems resemble each other in that there exists a negative feedback loop between a transcription factor and a chaperone. However, loops are organized such that TF is an activator in one and is an inhibitor in another. In the following, we will demonstrate how this difference leads to the distinctly different binding affinities of TF to chaperones.

The constraints on binding affinities can be understood when we look at how TF and chaperones are related in the steady state. From equations (1), (4) and (6) we obtain the expression for free σ^{32} to be

$$[\sigma^f] = \frac{\eta K_j}{\gamma_s K_j + (\gamma_c + \gamma_s)[D^f]} = \frac{\eta/\gamma_s}{1 + \dfrac{[D^f]}{\gamma_s K_j/(\gamma_c + \gamma_s)}}. \quad (17)$$

This is a decreasing function of the free chaperone $[D^f]$, and $[\sigma^f]$ approaches a constant value η/γ_s when $[D^f] \ll \gamma_s K_j/(\gamma_c+\gamma_s)$. Here, $\gamma_s K_j/(\gamma_c+\gamma_s) \sim 3$ as we fix to $\gamma_s = 0.03$ and $K_j = 100$ based on experimental observations.

Similarly for the HrcA, from equations (9), (12) and (14), we find

$$[H^a] = \frac{\beta_h [H^t][G^f]}{\gamma_c K_l + (\gamma_c + \beta_h)[G^f]} = \frac{\beta_h [H^t][G^f]/\gamma_c K_l}{1 + \dfrac{[G^f]}{\gamma_c K_l/(\gamma_c + \beta_h)}}. \quad (18)$$

This is an increasing function of the free chaperone $[G^f]$, and $[H^a]$ approaches a constant value $\beta_h[H^t]/(\gamma_c + \beta_h)$ when $[G^f] \gg \gamma_c K_l/(\gamma_c+\beta_h)$. Note that both K_l and β_h are unknown parameters for the HrcA system.

These expressions (with parameters from table 1) are plotted in figure 4(*A*) as functions of free chaperones. As chaperones activate HrcA and inhibit σ^{32}, HrcA is increasing and σ^{32} is decreasing with increasing amounts of free chaperones. Each of the curves has two characteristic regimes. (a) *Insensitive regime* where TF is insensitive to changes in chaperone concentration. This corresponds to a nearly flat region in the plot, where concentration of TF does not depend or depends very weakly on the chaperone concentration (chaperones< 3 for σ^{32}, and chaperones$> 10^5$ for HrcA) (b) *Sensitive regime* where a change in the chaperone concentration results in a change in the TF concentration (chaperones> 3 for σ^{32}, and chaperones$< 10^5$ for HrcA).

For the system to be responsive and adjust the production rate of chaperones (controlled by amounts of active TFs) in response to changes in unfolded proteins (reflected by the amounts of free chaperones), it is essential for the system to function within sensitive regimes. The peculiar feature of the two systems is that the insensitive regimes lie in the opposite ends of chaperone concentrations; the σ^{32} system is sensitive as long as the free chaperone $[D^f]$ is *above* the threshold concentration, while the HrcA system can work as long as the free chaperone $[G^f]$ is *below* the threshold concentration. At the same time, the maximal concentration of free chaperone is limited by the amount of total proteins ($\sim 20\,000$), and the minimum represents the case when all chaperones are bound to unfolded proteins. As in the steady state the amounts of free chaperones vary between 5000–10 000, it is crucial that the sensitive regime spans this range. As the insensitive regime for σ^{32} lies in the range of small concentrations (threshold $\gamma_s K_j/(\gamma_c + \gamma_s) \sim 3$ with experimentally evaluated parameters), it will always be in the sensitive range. In contrast, in the HrcA system, the threshold must be larger than the typical steady state concentrations of free chaperones, i.e. $\gamma_c K_l/(\gamma_c + \beta_h) > 10\,000$. This impose the condition on binding affinity to be large enough, $K_l > 10\,000$, because the threshold value mainly depends on K_l and tuning other parameters such as β_h or $[H^t]$ does not affect threshold values much (figures 4(*B*) and (*C*)).

It now becomes clear why we could not obtain the responses in HrcA with $K_l \sim K_j \sim 100$, as this tight binding in the HrcA system would decrease the sensitivity regime to be below 100 nM or below typical steady state concentrations of free chaperones. For a similar reason, we cannot use weak binding affinity for σ^{32}, i.e. $K_j = 100\,000$, as it will shrink the sensitivity region to be above 3000 nM.

Alternatively, if in reality $K_l \sim 100$ nM, then this would imply that the sensitivity regime is very narrow, which means that the steady states of free chaperones have to vary between 1 and 100 nM. In principle, this could be the case, but this would imply that the system is not robust to sudden increases in unfolded proteins.

This difference in the chaperone–TF dissociation constant between the two systems is very critical. While both systems have a negative feedback as a core regulatory mechanism, our model predicts that the details of how each of the feedback is realized result in very different dissociation constants. One can test this prediction experimentally by varying binding affinity

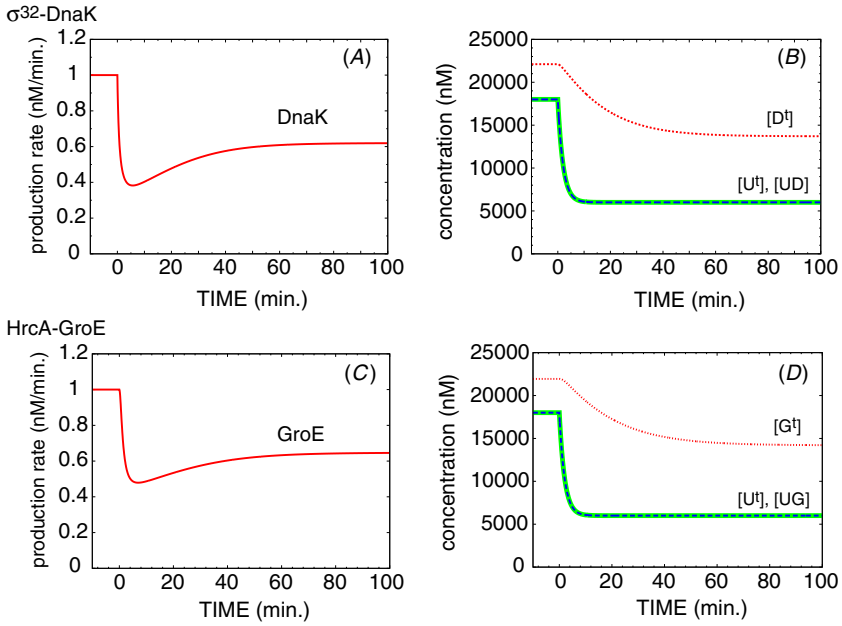

Figure 3. Inverse heat shock response in the σ^{32}–DnaK system ((*A*) and (*B*)) and the HrcA–GroE system ((*C*) and (*D*)). (*A*) and (*C*) shows the time evolution of chaperone production rate normalized by the pre-stimulus level. ((*B*) and (*D*)) shows the time evolution of the density of total chaperones (dotted lines), total unfolded-protein (bold lines), and complex formed by chaperone and unfolded proteins (broken lines). Parameters used in simulations are shown in table 1 except for production rate of unfolded proteins, $F(T)$, which decreases at $t = 0$, $F(T) = 9000 \rightarrow 3000$.

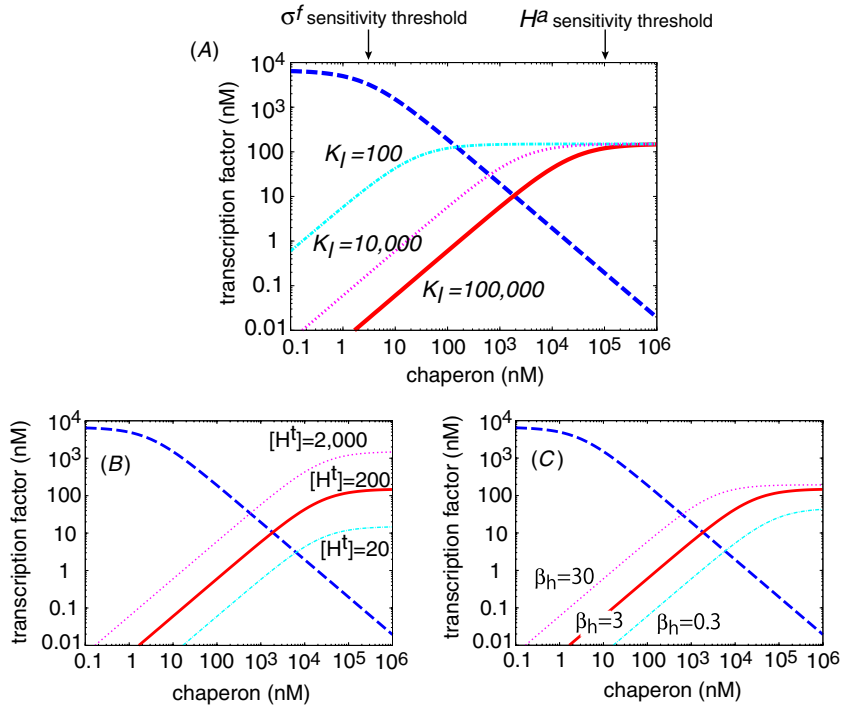

Figure 4. Relation between the free chaperone and transcription factor in a steady state given by equations (17) and (18). $[\sigma^f]$ is with the parameters in table 1 (bold broken line), while for $[H^a]$ some lines changing a parameter (K_l in A, $[H^t]$ in B and β_h in C) is shown, and $\beta_h = 3$, $[H^t] = 200$ and $K_l = 100\,000$ are used if not otherwise specified.

of HrcA to GroEL, and investigate how this affects response dynamics.

Parameter robustness

The results presented so far were based on a single set of parameters, chosen to reproduce experimental data. Some of them are fixed to a known experimental values as already mentioned in the model section, while the rest of the parameters are fitted to reproduce heat shock dynamics; there are five fitting parameters for the σ^{32}–DnaK system and seven for HrcA–GroE (see table 1). To understand how constrained our parameter choice is, we studied robustness of parameters.

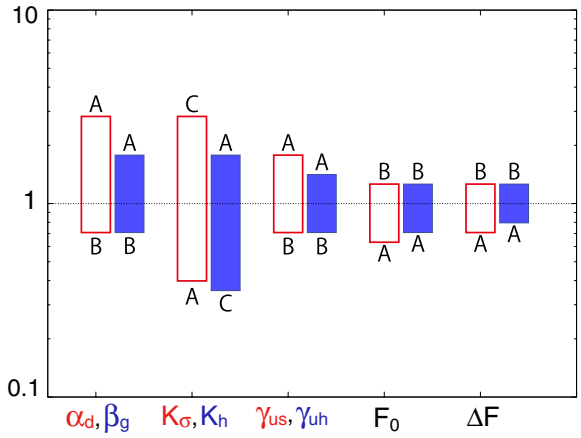

Figure 5. Robustness of the model against parameter changes. For each parameter (horizontal axis), fold change (vertical axis) of the value from the reference value in table 1 is tried, and the range of the parameter that is able to reproduce a reasonable heat shock response (defined by conditions (i)–(iv)) is shown. Red open bars show the results for the σ^{32}–DnaK system, and blue filled bars are for the HrcA–GroE system. F_0 denotes the initial value and ΔF denotes fold change in $F(T)$. Capital letters at the top and the bottom of the bars show which conditions ((*A*), (*B*) and (*C*)) are broken by further decrease (increase) in the corresponding parameter; (*A*) peak amplitude, (*B*) time and (*C*) recovering conditions in chaperone production rate (see the text for details).

Figure 5 shows how much a given parameter can be changed (the maximal fold of change from the values shown in table 1 with preserving proper heat shock response.

We choose a proper response to the one characterized by (*A*) a peak with more than two and less than ten fold change (normalized to the pre-stimulus level) (*B*) occurring within 10 time units and (*C*) recovering to a new steady state that is less than two fold of the pre-stimulus one in chaperone production rate. Interestingly, most parameters can only be changed by at most a few fold for both systems.

One of the main reasons why the system is rather sensitive to parameter choice is due to the importance of the stoichiometry between chaperones and unfolded proteins: if either chaperones or unfolded proteins are in excess there will be no peak. Excess of chaperones (e.g. due to high chaperone production rate α_d, β_g) will absorb a sudden increase in unfolded proteins, so that there will be no increase in chaperone production and thus no peak. On the other hand, excess of unfolded proteins (e.g. low α_d, β_g) will result in a state where chaperone production is maximally activated already before the shock. Thus, a further increase in unfolded proteins will not lead to the increase in production rate of chaperones.

Discussion

We have quantitatively investigated similarities and differences in two heat shock systems: one characteristic to gram negative bacteria (e.g. *E. coli*) and another to gram positive (e.g. *L. lactis*). Remarkably, although the two are very different at the level of promoter regulation, a striking similarity appears at the level of regulatory networks. Both are governed by chaperone-mediated negative feedback loops and in both cases chaperone sequestration is employed as a stress sensing mechanism. Furthermore, the similarity continues at the level of the response dynamics—both systems have characteristic rapid transient responses.

There are three core features characteristic to both systems that are necessary to generate a rapid transient response observed in both systems upon direct heat shock (a sudden increase in unfolded proteins).

- The initial rapid increase is governed by chaperone independent rates, which are the σ^{32} synthesis rate or the rate of HrcA conversion into an inactive form. The initial slope of increase in chaperones is governed by respectively η and γ_c.
- The rapid recovery to the steady state is governed by chaperone mediated processes (degradation of σ^{32} or activation of HrcA).
- The peak is the result of two rather different timescales involved: a rapid dynamics of TF (determining rapid increase and decrease) and a slow chaperone synthesis, determining the time of the peak in transient response, i.e. time when there are enough chaperones to deal with increased amounts of unfolded proteins.

Furthermore, different realizations of negative feedbacks—one through an transcriptional activator another through transcriptional inhibitor—impose distinct constraints on chaperone–TF binding affinities. Our analyses predict that whereas the tighter TF–chaperone binding increases the dynamic range for the σ^{32} system, it would work in an opposite direction and decrease the dynamic range for the HrcA system. In other words, chaperone–TF binding affinity imposes a lower limit on the amounts of free chaperones for the σ^{32} system, while it becomes the upper limit for the HrcA system. With the experimentally determined binding affinity for the σ^{32} system, the lower limit for free chaperones is 1 nM (see figure 4), i.e. one free chaperone per cell, which is low enough to account for possible variations in chaperone levels. (The upper bound in this case is determined by the amounts of TF to be not less than 1–2 protein per cell (1–2 nM) so that each cell feels change in TF, thus setting upper limit to about 10^4, see figure 4.)

We predict that the chaperone–HrcA binding should match the upper limit of the desired amount of free chaperones and when measured can thus serve as an indirect indication of the amounts of free chaperones in the cell.

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