Balance of ATPase stimulation and nucleotide exchange is not required for efficient refolding activity of the DnaK chaperone

ARTICLE *in* FEBS LETTERS · NOVEMBER 2005

Impact Factor: 3.17 · DOI: 10.1016/j.febslet.2005.09.056 · Source: PubMed

3 AUTHORS, INCLUDING:

Yvonne Groemping
Center of Advanced European Studies and …

21 PUBLICATIONS **977** CITATIONS

Balance of ATPase stimulation and nucleotide exchange is not required for efficient refolding activity of the DnaK chaperone

Yvonne Groemping, Ralf Seidel[1], Jochen Reinstein*

Max-Planck-Institute für medizinische Forschung, Abteilung Biomolekulare Mechanismen, Jahnstrasse 29, D-69120 Heidelberg, Germany

Received 28 July 2005; revised 22 September 2005; accepted 22 September 2005

Available online 5 October 2005

Edited by Felix Wieland

Abstract The DnaK system from *Thermus thermophilus* ($DnaK_{Tth}$) exhibits pronounced differences in organisation and regulation to its mesophile counterpart from *Escherichia coli* ($DnaK_{Eco}$). While the ATPase cycle of $DnaK_{Eco}$ is tightly regulated by the concerted action of the two cofactors $DnaJ_{Eco}$ and $GrpE_{Eco}$, the $DnaK_{Tth}$ system features an imbalance in this cochaperone mediated regulation. $GrpE_{Tth}$ considerably accelerates the ATP/ADP exchange, but $DnaJ_{Tth}$ only slightly stimulates ATPase activity, believed to be a key step for chaperone activity of $DnaK_{Eco}$. By in vitro complementation assays, we could not detect significant ATPase-stimulation of orthologous $DnaJ_{Tth} \cdot DnaK_{Eco}$ or $DnaJ_{Eco} \cdot DnaK_{Tth}$-complexes as compared to the $DnaK_{Eco}$ system, although they were nevertheless active in luciferase refolding experiments. Assistance of protein recovery by DnaK thus seems to be uncoupled of the magnitude of DnaJ mediated ATPase-stimulation.

Keywords: Chaperone; Folding; Kinetics; DnaK-system; Regulation

1. Introduction

Molecular chaperone systems assist protein folding by a variety of mechanisms. For one of the biochemically and structurally best characterised systems, Hsc70/DnaK [1,2], the major modes of action can be summarised as follows: The DnaK system is believed to act as an ATP driven cycling buffer that removes aggregation prone substrate proteins from free solution. The accessory co-chaperones DnaJ and GrpE mainly act as regulators of two opposing key switches that determine the nucleotide state and thus substrate binding properties of DnaK · GrpE accelerates nucleotide exchange considerably and populates the DnaK · ATP substrate scanning state, comparable to guanine nucleotide exchange factors [3–7], the activity of GrpE proteins being conserved among DnaK systems. The role of DnaJ is equivalent to the GTPase accelerating protein (GAP) in small G-protein systems since it stimulates the ATPase activity of DnaK from *Escherichia coli* by 3–4 orders of magnitude and thus favours the DnaK.ADP holding state [8–10]. DnaJ proteins are multidomain proteins: besides the J-domain that is essential for ATPase stimulation [11,12], a G/F- domain, a Zink-binding and a C-terminal domain are necessary for the $DnaJ_{Eco}$ protein to be active in refolding experiments [11,13]. It is believed that the G/F and the J-domain (together) are involved in the ATPase stimulation, both of which are present in the thermophile homolog $DnaJ_{Tth}$, with a sequence identity of 59% for the J-domains of $DnaJ_{Tth}$ and $DnaJ_{Eco}$. Nevertheless $DnaJ_{Tth}$ does only moderately stimulate hydrolysis with $DnaK_{Tth}$ in the absence of substrates, as described previously [14].

We characterised the ability of $DnaJ_{Tth}$ to stimulate ATP hydrolysis using single-turnover and steady-state ATPase assays with the homologous $DnaK_{Tth}$ system as well as in complementation with orthologous members of the DnaK system from *E. coli*.

We found no correlation of a pronounced ATPase stimulation with chaperone activity using renaturation of GdnHCl denatured firefly luciferase, a model system that was also used with the DnaK system from *E. coli* [15,16].

2. Experimental procedures

2.1. Protein purification

The molecular chaperones from *E. coli* and *Thermus thermophilus* were purified and depleted of intrinsic nucleotide essentially as described [4,14].

2.2. Single-turnover ATPase assays

Single-turnover ATPase assays were performed essentially as described [17] with 2 μM $DnaK_{Tth}$ or $DnaK_{Eco}$, 1.6 μM [γ^{32}P] ATP in 20 μl buffer (50 mM Tris/HCl pH 7.5, 100 mM KCl, 5 mM $MgCl_2$, 2 mM EDTA; 2 mM DTE) at 25 °C in the presence or absence of 2 μM luciferase and 2 μM $DnaJ_{Tth}$ or $DnaJ_{Eco}$. The assay contained 0.4 μM $GrpE_{Eco}$ or $GrpE_{Tth}$, respectively, to ensure that nucleotide binding is not rate limiting.

2.3. Steady-state ATPase assay

Steady-state ATP hydrolysis was measured using a colorimetric assay, where ATP is regenerated using 29 μg/ml lactate dehydrogenase and 71 μg/ml pyruvate kinase. The decrease in NADH absorption is used directly as a measure for ATP hydrolysis. Luciferase was denatured in unfolding buffer and diluted 1:125 in NADPH buffer (50 mM Tris/HCl, pH 7.5,

*Corresponding author. Fax: +49 6221 486585.
E-mail address: jochen.reinstein@mpimf-heidelberg.mpg.de (J. Reinstein).

[1] Max-Planck-Institut für molekulare Physiologie, Abteilung physikalische Biochemie, Otto-Hahn-Strasse 11, D-44227 Dortmund, Germany.

0014-5793/$30.00
doi:10.1016/j.febslet.2005.09.056

100 mM KCl, 5 mM $MgCl_2$, 2 mM EDTA, 2 mM DTE, 0.05 mg/ml BSA, 0.4 mM phosphoenolpyruvate, 0.2 mM NADH) with or without chaperones. The concentrations of proteins in NADH buffer were: [DnaK] = 3.2 μM; [GrpE] = 0.4 μM; [$DnaJ_{Eco}$] = 0.8 μM; [$DnaJ_{Tth}$] = 1.6 μM; [Luciferase] = 0.08 μM. The assay was performed at 25 °C.

2.4. Luciferase refolding in a continuous assay

Luciferase (Promega) was incubated for 2 min in unfolding buffer (25 mM HEPES/NaOH pH 7.5; 50 mM KCl; 15 mM $MgCl_2$; 2 mM DTE; 1 mM ATP; 0.05 mg/ml $BSA_{acetylated}$ and 5 M GdnHCl) and subsequently diluted 125-fold into refolding/assay buffer with 240 μM CoA and 0.1 mM luciferin. The refolding was measured continuously [18] at 30 °C using an Ascent Fluoroskan Fl spectrometer. The concentrations of chaperones contained in the refolding buffer were: [DnaK] = 3.2 μM; [GrpE] = 0.4 μM; [$DnaJ_{Eco}$] = 0.8 μM; [$DnaJ_{Tth}$] = 0.8 μM; [luciferase] = 0.08 μM. The yields of luciferase refolding was normalised to the yield of spontaneous refolding in the absence of chaperones.

3. Results

3.1. Single-turnover ATPase – determination of the maximal turnover rate

$DnaJ_{Tth}$ does not stimulate ATP hydrolysis significantly. Since the $DnaK_{Tth}$ system differs substantially from the *E. coli* Hsp 70 system in its genomic and oligomeric organisation, the question arises which of the key steps that were proposed to be essential for $DnaK_{Eco}$ function and regulation are preserved and essential in general. While GrpE mediated nucleotide exchange is conserved among the systems, $DnaJ_{Tth}$ in contrast to $DnaJ_{Eco}$ does not seem to accelerate ATP hydrolysis to a similar extent, the stimulation factor being 2–3 instead of 1500 [14,19]. To clarify if this is a specific feature of $DnaJ_{Tth}$ or if $DnaK_{Tth}$ itself can not be activated significantly, we performed single-turnover ATPase assays with orthologous complexes of the $DnaK_{Tth}$ and $DnaK_{Eco}$ chaperone systems (Fig. 1). In order to determine the effect of an unfolded substrate protein on the hydrolysis rate, we added denatured luciferase (under refolding conditions). In the case of $DnaJ_{Eco}$ the addition of a protein substrate was shown to further enhance the ATPase hydrolysis rate [7–9]. In the presence of catalytic amounts of GrpE these assays can detect DnaJ mediated ATPase stimulation with high sensitivity since they directly measure the rate of hydrolysis without being affected by the rates of ATP binding or ADP release. GrpE itself does not alter the ATPase activity, but accelerates nucleotide binding and release, which in the case of $DnaK_{Tth}$ are the rate limiting steps in the ATPase cycle [14,20].

$DnaJ_{Tth}$ stimulates the observed rate constant of $DnaK_{Tth}$ mediated ATP hydrolysis by a factor of 4, independent of the presence of luciferase. Likewise, the stimulation of $DnaK_{Eco}$s ATPase activity by $DnaJ_{Tth}$ is 3-fold and again the addition of luciferase does not show a significant difference. $DnaJ_{Eco}$ increases the hydrolysis rate of $DnaK_{Tth}$ by a factor of 5 or 6 in the absence or presence of luciferase. In comparison to the about 1500–15000-fold stimulation of $DnaK_{Eco}$ by $DnaJ_{Eco}$ [8,9] we conclude that there is no significant acceleration of ATPase activity by $DnaJ_{Tth}$ nor can $DnaK_{Tth}$ ATPase activity be enhanced more than 6-fold.

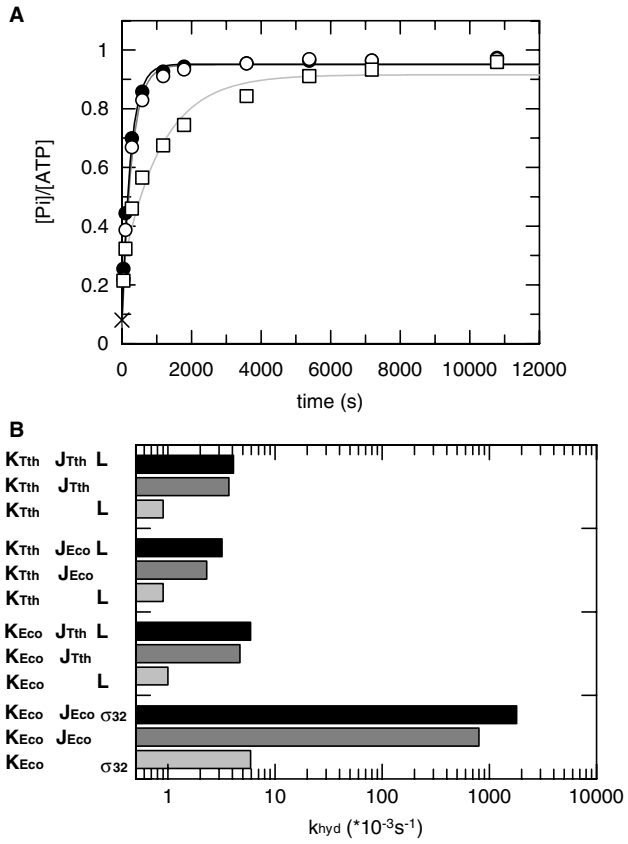

Fig. 1. Stimulation of ATPase activity by $DnaJ_{Tth}$ – single-turnover analyses. (A) The single-turnover ATPase activity of $DnaK_{Tth}$ was measured in the presence of denatured luciferase (□), $DnaJ_{Tth}$ (○) or $DnaJ_{Tth}$ and denatured luciferase (●) as described in Section 2. (×) control without enzyme. Curves drawn indicate the result of single exponential analyses with rate constants of $0.9 \times 10^{-3}\ s^{-1}$ (□), $3.7 \times 10^{-3}\ s^{-1}$ (○) and $4.1 \times 10^{-3}\ s^{-1}$ (●), respectively. (B) Comparison of single-turnover ATPase activity of the $DnaK_{Tth}$ and $DnaK_{Eco}$ systems. (a) Measurements of orthologous components of the $DnaK_{Tth}$ and $DnaK_{Eco}$ systems with luciferase as substrate (L). (b) Data adapted from [9] for homologous $DnaK_{Eco}$ system (quenched flow experiments with transcription factor σ^{32} as substrate).

The hydrolysis rate of DnaK alone (not shown) did not change in the presence of substrates. Luciferase was not used with $DnaK_{Eco}$ because of solubility problems at the high concentrations needed with a quenched flow experiment (as the hydrolysis rate of $DnaK_{Eco}$ in the presence of $DnaJ_{Eco}$ is too fast to be studied using hand mixing experiments and subsequent thin layer chromatography). Therefore we compared the rates of the two systems with the aid of a steady-state experiment. Under these conditions a direct comparison between the two systems is possible.

3.2. Steady-state ATPase – $DnaJ_{Tth}$ slightly changes the hydrolysis rate for both DnaK proteins under refolding conditions in the presence of substrate

Steady-state assays were performed in the presence and absence of denatured luciferase to compare the ATPase rate of the chaperone system under conditions used in the refolding assay. GrpE was always present in these experiments to ensure nucleotide binding and release are not limiting, as for the thermophile system binding and release of nucleotides are actually slower than the hydrolysis itself [21]. The hydrolysis rates for

$DnaK_{Tth}$ and $DnaK_{Eco}$, normalised to DnaK concentrations, are shown in Fig. 2A and B, respectively. Stimulation of $DnaK_{Tth}$ ATPase activity by $DnaJ_{Tth}$ is marginal, about 1.4-fold. This factor is not altered by addition of luciferase, up to a concentration of 0.32 μM (four times the concentration of luciferase in refolding assays). $DnaJ_{Eco}$ seems to moderately accelerate the ATPase rate of $DnaK_{Tth}$, 3-fold and if luciferase is added this factor increases to a 7-fold stimulation. In combination with $DnaK_{Eco}$, $DnaJ_{Eco}$ leads to an increase in hydrolysis rate by more than 30-fold, further enhanced by addition of the substrate (factor of 40). $DnaJ_{Tth}$, however, is barely able to stimulate the hydrolysis rate of $DnaK_{Eco}$ under these conditions, in the presence or absence of substrate (factor 2–3). These experiments show that enhancement of ATPase activity of both DnaK proteins by $DnaJ_{Tth}$ under conditions used for the refolding of substrate proteins is magnitudes lower than with $DnaJ_{Eco}$.

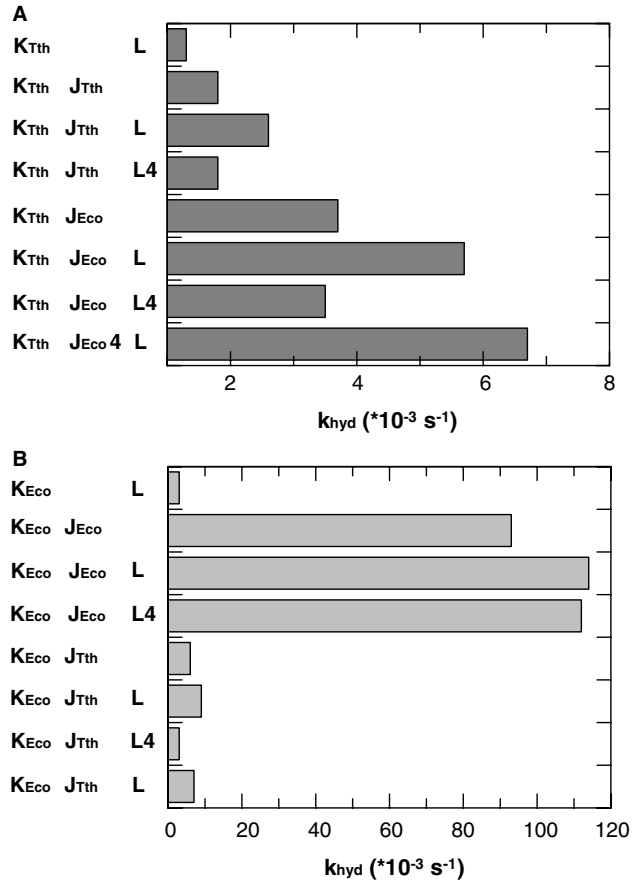

Fig. 2. Stimulation of DnaK ATPase activity by $DnaJ_{Tth}$ – steady-state analyses. Stimulation of ATPase activity of DnaK form *E. coli* and *T. thermophilus* with different DnaJ proteins was measured using a colorimetric assay in the presence (or absence) of GdnHCl-denatured luciferase as described in Section 2. The corresponding GrpE proteins were present to allow fast binding and release of nucleotides. (A) $DnaK_{Tth}$ (K_{Tth}) based complexes 3.2 μM $DnaK_{Tth}$ (K_{Tth}); 0.8 μM $DnaJ_{Tth}$ (J_{Tth}); 3.2 μM $DnaJ_{Tth}$ ($J_{Tth}4$); 0.4 μM $GrpE_{Tth}$; 0.8 μM $DnaJ_{Eco}$ (J_{Eco}); 3.2 μM $DnaJ_{Eco}$ ($J_{Eco}4$); 0.08 μM luciferase (L); 0.32 μM luciferase (L4). (B) $DnaK_{Eco}$ (K_{Eco}) based complexes 3.2 μM $DnaK_{Eco}$ (K_{Eco}); 0.8 μM $DnaJ_{Eco}$ (J_{Eco}); 3.2 μM $DnaJ_{Eco}$ ($J_{Eco}4$); 0.4 μM $GrpE_{Eco}$; 0.8 μM $DnaJ_{Tth}$ (J_{Tth}); 3.2 μM $DnaJ_{Tth}$ ($J_{Tth}4$); 0.08 μM luciferase (L); 0.32 μM luciferase (L4).

3.3. Luciferase refolding in the presence of orthologous chaperone complexes

Efficient refolding is possible in the absence of significant ATPase stimulation. The $DnaK_{Tth}$ system, albeit originating from an organism that lives at about 75 °C, is fully functional at 30 °C in a continuous luciferase refolding assay as described previously [19]. Although the $DnaK_{Tth}$ chaperone cycle is slightly slower than $DnaK_{Eco}$ the maximal yield of active luciferase appears to be comparable in both systems. In this context it might be interesting to note that the ATPase activity of $DnaK_{Tth}$ is hardly temperature dependent and changes only 10-fold between 25 and 75 °C, with an activation energy E_a of 25.7 kJ mol^{-1} [21].

A balance of cochaperone action is not essential for DnaK refolding activity – since DnaJ in neither case stimulates ATPase activity of DnaK more than several fold, the question arises whether pronounced DnaJ stimulated ATP hydrolysis is necessary for the refolding of luciferase, as this step is considered to be essential for trapping of substrate proteins in *E. coli* [8,9,22]. The GrpE proteins of both organisms were shown previously to complement in nucleotide exchange assays [19]. Nucleotide exchange rates with $DnaK_{Eco}$/$DnaK_{Tth}$ and $GrpE_{Tth}$ are equivalent which is reflected in their comparable refolding times. $GrpE_{Eco}$ complements less efficiently and the lower exchange activities lead to a slower refolding of luciferase. We therefore substituted the co-chaperones GrpE and DnaJ from *T. thermophilus* with their corresponding *E. coli* counterparts in luciferase refolding assays. In the absence of DnaK, DnaJ or GrpE, luciferase refolding is only marginal (Fig. 3A and B). In the presence of the $DnaK_{Tth}$ chaperones the yield of refolded enzyme is 16-fold higher than without any chaperones. If $DnaJ_{Tth}$ is used at the same concentration instead of $DnaJ_{Eco}$ the yield drops to a very low level (data not shown), similar to that without chaperones. However, with increasing $DnaJ_{Tth}$ concentrations (Fig. 3B, $DnaJ_{Tth}4$) the yield increases to a value similar to that observed with $DnaJ_{Eco}$, indicating that $DnaJ_{Tth}$ has a somewhat lower affinity for $DnaK_{Eco}$ but is still functional. Although $DnaJ_{Tth}$ does not stimulate ATP hydrolysis appreciably, it seems to be important for the refolding of denatured proteins.

4. Discussion

Although the two DnaK systems from *T. thermophilus* and *E. coli* are highly homologous, they show pronounced differences in organisation and regulation. The ATPase cycle of the chaperone DnaK is regulated by the two cochaperones DnaJ and GrpE as illustrated in Fig. 4. A balanced function of both cochaperones was assumed to be crucial for proper chaperone function [4,23]. In the DnaK system from *E. coli* this is achieved by a 1500–15000-fold stimulation of the ATPase rate by DnaJ and a 5000-fold acceleration of nucleotide exchange by GrpE (Fig. 4A). Since the intrinsic rates of ATP hydrolysis are also comparable in magnitude, this results in a sensitive balance of the ATP- and ADP-states of DnaK and thus substrate binding and release properties. For $DnaK_{Tth}$ however (Fig. 4B) it was shown that $GrpE_{Tth}$ significantly enhances nucleotide exchange by a factor of 80000 while $DnaJ_{Tth}$ seemed to be less functional, enhancing the ATPase rate only 3–4-fold which leads to an apparent imbalance in the presence of regulatory proteins. The dnaK-operon from *T. thermophilus* also codes for the co-chaperone $DafA_{Tth}$, which appeared to

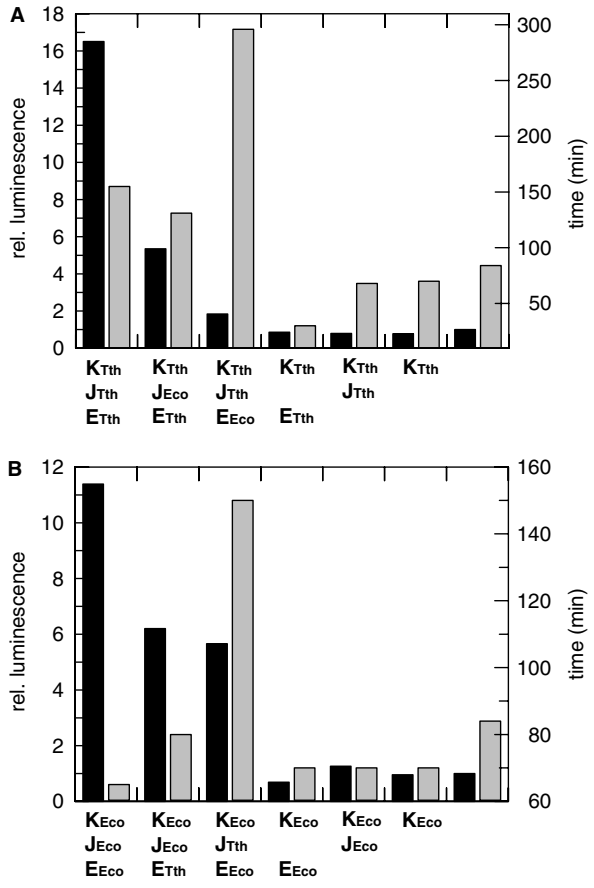

Fig. 3. $DnaJ_{Tth}$ is an essential component of the $DnaK_{Tth}$ chaperone system – despite the lack of significant ATPase stimulation. Different combinations of the components of the *E. coli* and *T. thermophilus* DnaK chaperone systems were assayed for chaperone activity as described in Section 2. The maximal yield of renatured luciferase obtained is indicated with black bar charts (left axis) and the corresponding time where this maximum was achieved with grey bar charts (right axis). In all cases, the presence of DnaJ is necessary for efficient chaperone activity. The protein concentrations were [DnaJ] = 0.8 or 3.2 μM (DnaJ4); [DnaK] = 3.2 μM; [GrpE] = 0.4 μM and [Luciferase] = 0.08 μM. The luminescence of luciferase refolded in the absence of chaperones was set to 1 and the refolding yields in the presence of chaperones was normalised to the yield of spontaneously refolded luciferase. (A) $DnaK_{Tth}$ (K_{Tth}) based system and (B) $DnaK_{Eco}$ (K_{Eco}) based system.

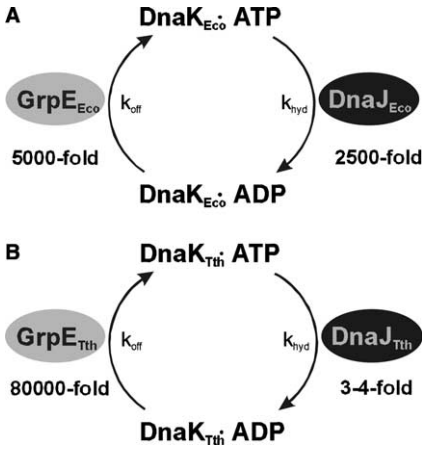

Fig. 4. Comparison of key constants for the $DnaK_{Eco}$ and $DnaK_{Tth}$ nucleotide cycles. The figure shows the basic nucleotide cycle for the ATPase DnaK. Nucleotide exchange is efficiently catalysed by the GrpE proteins from both organisms. The thermophile exchange factor enhances the ADP release by a factor of 80 000, about 10 times more than the *E. coli* homolog. This acceleration is crucial for the thermophile system as nucleotide binding and release are the rate-limiting steps in this cycle, being slower than the actual hydrolysis. ATP hydrolysis is stimulated 2500-fold for the *E. coli* chaperone system, while in the case of *T. thermophilus* an effective refolding of substrates can be achieved despite of an only 3–4-fold acceleration of the ATPase rate.

be unique to the thermophile system. This factor mediates the formation of a stable, high-affinity heterononameric complex with $DnaK_{Tth}$ and $DnaJ_{Tth}$ [14,24] and leads to the dissociation of peptide from a $DnaK_{Tth}$ · peptide complex. A remote relative of $DafA_{Tth}$, CbpM was recently reported for the DnaK system of *Escherichia coli* [25].

The formation of a stable complex between DnaK proteins and their corresponding DnaJs is rather exceptional. In the very well characterised DnaK system from *E. coli*, $DnaK_{Eco}$ and $DnaJ_{Eco}$ only interact temporarily with a weak affinity and 1:2 stoichiometry ($DnaK_{Eco}$:$DnaJ_{Eco}$) [26]. However one additional example for high affinity DnaK · DnaJ complexes was reported previously. The yeast ribosome-associated-complex (RAC) consists of the DnaK homolog Ssz1p and the DnaJ homolog Zuotin. They interact tightly, in a 1:1 stoichiometry either in the presence of ATP or ADP, in analogy to the $DnaK_{Tth}$ · $DnaJ_{Tth}$ complex [27]. Another similarity between RAC and the thermophile system is the absence of a significant ATPase activation. Both Zuotin [27] and $DnaJ_{Tth}$ accelerated hydrolysis only marginally compared to $DnaJ_{Eco}$, where significant stimulation of ATPase activity is believed to be a key step for the "locking in" of substrates [9,28,29].

Since $DnaJ_{Tth}$ lacks a Zink-binding domain that was found with many DnaJ proteins it might be attractive to connect the missing ATPase stimulation to that domain. However in the yeast cytosol two different DnaJ proteins coexist that both interact with the same Hsp70 protein. One of them, Ydj1, contains a Zink-binding domain, whereas the other, Sis1, does not. Both are active in luciferase refolding assays and stimulate the ATPase rate of the corresponding Hsp70 protein 11 and 8-fold in steady-state ATPase assays (Ydj1 and Sis1, respectively), [30] supporting the view that the Zn^{2+}-domain of DnaJ is not involved in ATPase activation but rather substrate specificity. Furthermore it was shown removal of the Zn-binding domain in DnaJ did not influence the ATPase stimulation activity [10,13,31]. Although most of the DnaJ proteins are not kinetically characterised in detail, it appears as if $DnaJ_{Eco}$ mediated activation of ATP hydrolysis by 3–4 orders of magnitude is rather exceptional. This raises the question whether such a pronounced stimulation is a general characteristic of DnaJ proteins or rather represents a specific adaptation of the *E. coli* protein to its environment and the volatile nature of the $DnaK_{Eco}$ · $DnaJ_{Eco}$ complex. In the case of *E. coli* the stimulation of ATPase is related to the presence of substrates. Substrate proteins but not peptides enhance the hydrolysis rate and DnaJ binds protein substrates on its own, targeting them to $DnaK_{Eco}$. The availability of substrates is thus coupled to the ATPase stimulation leading to the locking in of substrates. In *T. thermophilus* this coupling does not exist, as the stimulation of ATPase by the DnaJ-protein is only marginal. Here specificity may arise by the $DnaK_{Tth}$ · $DnaJ_{Tth}$ complex preas-

sembled by the small cofactor $DafA_{Tth}$, that upon heat shock is released by competing substrate proteins and can subsequently perform regulatory roles at the site of protein synthesis [32].

Both modes of coupling represent different strategies to achieve specificity for protein substrates; they may be adapted to the requirements of different environmental conditions. Substantial acceleration of ATPase activity could be an adaptation of the DnaJ protein from *E. coli* to the rather volatile nature of the $DnaK_{Eco} \cdot DnaJ_{Eco}$ complex, but does not seem to be an essential feature of all Hsp70/DnaK systems.

Acknowledgements: We are indebted to Ilme Schlichting and Roger S. Goody for continuous support, Petra Herde and Marion Hülseweh for excellent technical assistance and Bernd Bukau for providing overexpressing clones of $DnaK_{Eco}$, $GrpE_{Eco}$ and $DnaJ_{Eco}$. We also thank Tobias Restle, Katrin Rittinger and Simone Popp for critically reading of the manuscript. This work was supported by a grant of the Deutsche Forschungsgemeinschaft (DFG) to J.R. (SFB394, 1212/1- 1).

References

[1] Bukau, B. and Horwich, A.L. (1998) The hsp70 and hsp60 chaperone machines. Cell 92, 351–366.
[2] Agashe, V.R. and Hartl, F. (2000) Roles of molecular chaperones in cytoplasmic protein folding. Semin. Cell Dev. Biol. 11, 15–25.
[3] Liberek, K., Marszalek, J., Ang, D., Georgopoulos, C. and Zylicz, M. (1991) *Escherichia coli* DnaJ and GrpE heat shock proteins jointly stimulate ATPase activity of DnaK. Proc. Natl. Acad. Sci. USA 88, 2874–2878.
[4] Packschies, L., Theyssen, H., Buchberger, A., Bukau, B., Goody, R.S. and Reinstein, J. (1997) GrpE accelerates nucleotide exchange of the molecular chaperone DnaK with an associative displacement mechanism. Biochemistry 36, 3417–3422.
[5] Harrison, C.J., Hayer-Hartl, M., Di Liberto, M., Hartl, F.U. and Kuriyan, J. (1997) Crystal-structure of the nucleotide exchange factor GrpE bound to the ATPase domain of the molecular chaperone DnaK. Science 276, 431–435.
[6] Sondermann, H., Scheufler, C., Schneider, C., Höhfeld, J., Hartl, F.U. and Moarefi, I. (2001) Structure of a Bag/Hsc70 complex: convergent functional evolution of Hsp70 nucleotide exchange factors. Science 291, 1553–1557.
[7] Pierpaoli, E.V., Sandmeier, E., Schönfeld, H.-J. and Christen, P. (1998) Control of the DnaK chaperone cycle by substoichiometric concentrations of the co-chaperones DnaJ and GrpE. J. Biol. Chem. 273, 6643–6649.
[8] Russell, R., Karzai, A.W., Mehl, A.F. and McMacken, R. (1999) DnaJ dramatically stimulates ATP hydrolysis by DnaK: insight into targeting of Hsp70 proteins to polypeptide substrates. Biochemistry 38, 4165–4176.
[9] Laufen, T., Mayer, M.P., Beisel, C., Klostermeier, D., Mogk, A., Reinstein, J. and Bukau, B. (1999) Mechanism of regulation of Hsp70 chaperones by DnaJ cochaperones. Proc. Natl. Acad. Sci. USA 96, 5452–5457.
[10] Wittung-Stafshede, P., Guidry, J., Horne, B.E. and Landry, S.J. (2003) The J-domain of Hsp40 couples ATP hydrolysis to substrate capture in Hsp70. Biochemistry 42, 4937–4944.
[11] Wall, D., Zylicz, M. and Georgopoulos, C. (1994) The NH_2-terminal 108 amino acids of the *Escherichia coli* DnaJ protein stimulate the ATPase activity of DnaK and are sufficient for lambda replication. J. Biol. Chem. 269, 5446–5451.
[12] Cyr, D.M., Langer, T. and Douglas, M.G. (1994) DnaJ-like proteins: molecular chaperones and specific regulators of Hsp70. Trends Biochem. Sci. 19, 176–181.
[13] Karzai, A.W. and McMacken, R. (1996) A bipartite signaling mechanism involved in DnaJ-mediated activation of the *Escherichia coli* DnaK protein. J. Biol. Chem. 271, 11236–11246.
[14] Klostermeier, D., Seidel, R. and Reinstein, J. (1999) The functional cycle and regulation of the *Thermus thermophilus* DnaK chaperone system. J. Mol. Biol. 287, 511–525.
[15] Schröder, H., Langer, T., Hartl, F.U. and Bukau, B. (1993) DnaK, DnaJ and GrpE form a cellular chaperone machinery capable of repairing heat-induced protein damage. EMBO J. 12, 4137–4144.
[16] Szabo, A., Langer, T., Schröder, H., Flanagan, J., Bukau, B. and Hartl, F.U. (1994) The ATP hydrolysis-dependent reaction cycle of the *Escherichia coli* Hsp70 system – DnaK, DnaJ, and GrpE. Proc. Natl. Acad. Sci. USA 91, 10345–10349.
[17] Theyssen, H., Schuster, H.-P., Packschies, L., Bukau, B. and Reinstein, J. (1996) The second step of ATP binding to DnaK induces peptide release. J. Mol. Biol. 263, 657–670.
[18] Kolb, V.A., Makeyev, E.V. and Spirin, A.S. (1994) Folding of firefly luciferase during translation in a cell-free system. EMBO J. 13, 3631–3637.
[19] Groemping, Y., Klostermeier, D., Herrmann, C., Veit, T., Seidel, R. and Reinstein, J. (2001) Regulation of ATPase and chaperone cycle of DnaK from *Thermus thermophilus* by the nucleotide exchange factor GrpE. J. Mol. Biol. 305, 1173–1183.
[20] Groemping, Y. and Reinstein, J. (2001) Folding properties of the nucleotide exchange factor GrpE from *Thermus thermophilus*: GrpE is a thermosensor that mediates heat shock response. J. Mol. Biol. 314, 167–178.
[21] Klostermeier, D., Seidel, R. and Reinstein, J. (1998) Functional properties of the molecular chaperone DnaK from *Thermus thermophilus*. J. Mol. Biol. 279, 841–853.
[22] Han, W. and Christen, P. (2004) *cis*-Effect of DnaJ on DnaK in ternary complexes with chimeric DnaK/DnaJ-binding peptides. FEBS Lett. 563, 146–150.
[23] Brehmer, D., Rüdiger, S., Gassler, C.S., Klostermeier, D., Packschies, L., Reinstein, J., Mayer, M.P. and Bukau, B. (2001) Tuning of chaperone activity of Hsp70 proteins by modulation of nucleotide exchange. Nat. Struct. Biol. 8, 427–432.
[24] Motohashi, K., Yohda, M., Endo, I. and Yoshida, M. (1996) A novel factor required for the assembly of the DnaK and DnaJ chaperones of *Thermus thermophilus*. J. Biol. Chem. 271, 17343–17348.
[25] Chae, C., Sharma, S., Hoskins, J.R. and Wickner, S. (2004) CbpA, a DnaJ homolog, is a DnaK co-chaperone, and its activity is modulated by CbpM. J. Biol. Chem. 279, 33147–33153.
[26] Schönfeld, H.-J. and Behlke, J. (1998) Molecular chaperones and their interactions investigated by analytical ultracentrifugation and other methodologies. Methods Enzymol. 290, 269–296.
[27] Gautschi, M., Lilie, H., Funfschilling, U., Mun, A., Ross, S., Lithgow, T., Rucknagel, P. and Rospert, S. (2001) RAC, a stable ribosome-associated complex in yeast formed by the DnaK–DnaJ homologs Ssz1p and zuotin. Proc. Natl. Acad. Sci. USA 98, 3762–3767.
[28] Russell, R., Jordan, R. and McMacken, R. (1998) Kinetic characterization of the ATPase cycle of the DnaK molecular chaperone. Biochemistry 37, 596–607.
[29] Jordan, R. and McMacken, R. (1995) Modulation of the ATPase activity of the molecular chaperone DnaK by peptides and the DnaJ and GrpE heat shock proteins. J. Biol. Chem. 270, 4563–4569.
[30] Lu, Z. and Cyr, D.M. (1998) The conserved carboxyl-terminus and zinc finger-like domain of the co-chaperone Ydj1 assist Hsp70 in protein-folding. J. Biol. Chem. 273, 5970–5978.
[31] Linke, K., Wolfram, T., Bussemer, J. and Jakob, U. (2003) The roles of the two zinc binding sites in DnaJ. J. Biol. Chem. 278, 44457–44466.
[32] Dumitru, G.L., Groemping, Y., Klostermeier, D., Restle, T., Deuerling, E. and Reinstein, J. (2004) DafA cycles between the DnaK chaperone system and translational machinery. J. Mol. Biol. 339, 1179–1189.