Functional adaptations of the bacterial chaperone trigger factor to extreme environmental temperatures

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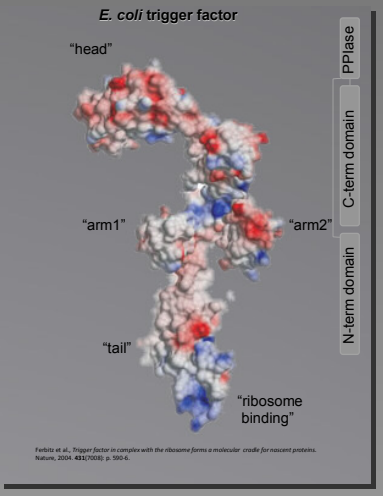
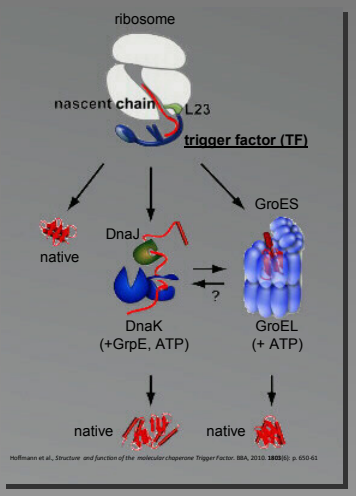
Comparative study of three trigger factors from different thermal origins

A key determinant of protein adaptation to temperature is the acquisition of the final, biologically active conformation aided by chaperones and folding catalysts, which remains almost unexplored.
The aim of this work was to identify the functional adaptations that enable trigger factor (TF) to be active in the wide range of biological temperatures. As TF is the first molecular chaperone interacting with virtually all nascent polypeptides synthesized by the bacterial ribosome and also possesses a PPIase activity, it represents a suitable model for this study. To cover nearly all temperatures encountered by living organisms, we compared three structurally homologous TFs.

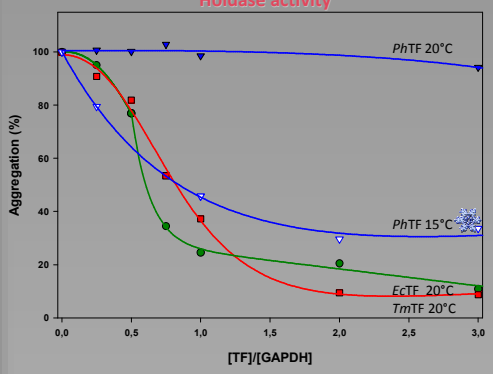
Protein	Source	Estimated environ. T°	Proteomic context
*Ph*TF	*P. haloplanktis* TAC125	< 0°C	Cold Acclimation Protein
*Ec*TF	*E. coli* RR1	37°C	Cold Shock Protein
*Tm*TF	*T. maritima* DSM3109	85-90°C	undetermined





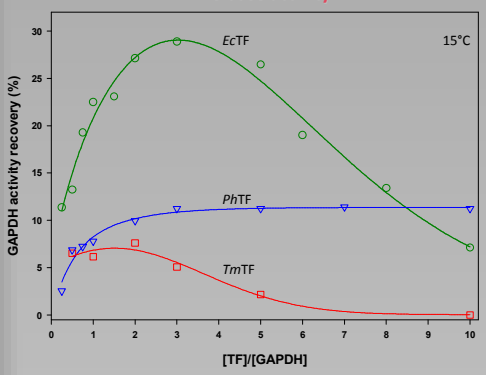
Chaperone activities of TFs

Holdase activity



Effects of TFs on GdmCl-denatured D-glyceraldehyde-3-phosphate dehydrogenase aggregation:
***Ec*TF and *Tm*TF -** Gradual prevention of aggregation at 20°C
***Ph*TF** - No protection against aggregation at 20°C, holdase activity maintained for 10-20 min at 15°C after cold incubation

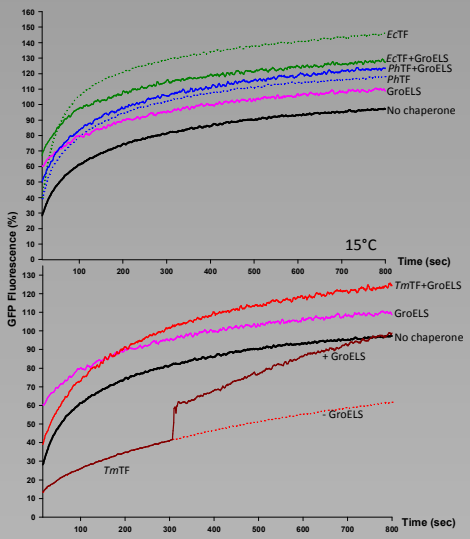
Foldase activity



Effects on the reactivation of GdmCl-denatured GAPDH:
***Ph*TF** - Moderate foldase activity
***Ec*TF** - Good foldase activity at low [TF], inhibition at high [TF]
***Tm*TF** - Inhibition

Foldase activity: effect of the experimental temperature

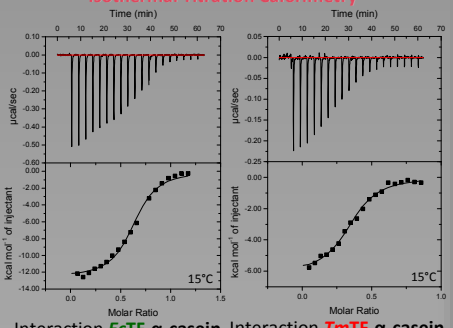
Acid-denatured green fluorescent protein refolding assay performed at different temperatures:
➢*Physiological T° (5°C for PhTF, 37°C for EcTF, 50°C for TmTF): consistent with GAPDH reactivation assay*
➢*15°C: similar results except for EcTF (no inhibition at high [TF])* → *consistent with its role of cold shock protein*



Cooperation of TFs with the chaperonin GroEL/ES+ATP:
GroELS - Improvement of GFP refolding yield
***Ec*TF+GroELS -** Competition between the 2 chaperones, *Ec*TF is a more effective foldase
***Ph*TF+GroELS -** Additive effects, independent catalysis of refolding
***Tm*TF+GroELS -** Folding recovery, cooperative effect highlighting a sequential action of chaperones, *Tm*TF as holdase and GroELS as foldase

Interaction TFs-unfolded protein

Isothermal Titration Calorimetry

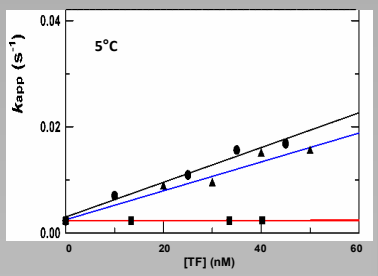


Interaction ***Ec*TF-α-casein** Interaction ***Tm*TF-α-casein**

***Ec*TF** – H-bonds and VDW interactions with the natively unstructured substrate
***Tm*TF** - Dominant hydrophobic effect in α-casein binding, confirmed by 8-Anilinonaphthalene-1-sulfonic acid titration, up to 4 *Tm*TF bound to 1 α-casein, corroborating its main role of holdase
***Ph*TF** - Very low affinity for non-native proteins

PPIase activity

Peptide and protein substrate assays



Catalytic efficiency:
***Ec*TF** - The most effective
***Ph*TF** - Similar to *Ec*TF at low temperature → PPIase function not adapted
***Tm*TF** - Very weak PPIase activity

Conclusions

Adaptations of the chaperone function

➢In *P. haloplanktis*, only *Ph*TF and GroELS are chaperones expressed significantly at low temperature and they catalyze protein folding independently. As cold prevents misfolding and aggregation, *Ph*TF is a weak foldase *in vitro*. It probably only retains its basic function of foldase associated with the ribosome.
➢*Ec*TF possesses a complete chaperone activity, essential at *E. coli* biological temperature which promotes misfolding and aggregation. As two efficient foldases, *Ec*TF and GroELS compete for the binding of the substrate. At low temperature and high [*Ec*TF], foldase activity persists, which is consistent with its role of cold shock protein.
➢*Tm*TF acts mainly as holdase, which can be related to the hydrophobicity of its chaperone cavity. Its cooperation with GroELS reveals that *Tm*TF forms a complex with proteins to protect them from high environmental temperatures that promote aggregation, before the transfer of the substrate to downstream chaperones for folding.

Adaptations of the PPIase function

➢*Ph*TF PPIase function is not specially adapted to cold. However, *P. haloplanktis* genome possesses 14 PPIases, and *Ph*TF is largely overexpressed (~40 x) at low temperature, which constitutes a peculiar adaptation of the PPIase function.
➢*E. coli* possesses 8 PPIases and needs a highly active TF because prolyl isomerisation is a rate-limiting step for protein folding at 37°C.
➢*Tm*TF is a weak PPIase, probably because prolyl isomerisation is not limiting at high temperature, as exemplified by the unique PPIase found in *T. maritima* genome.