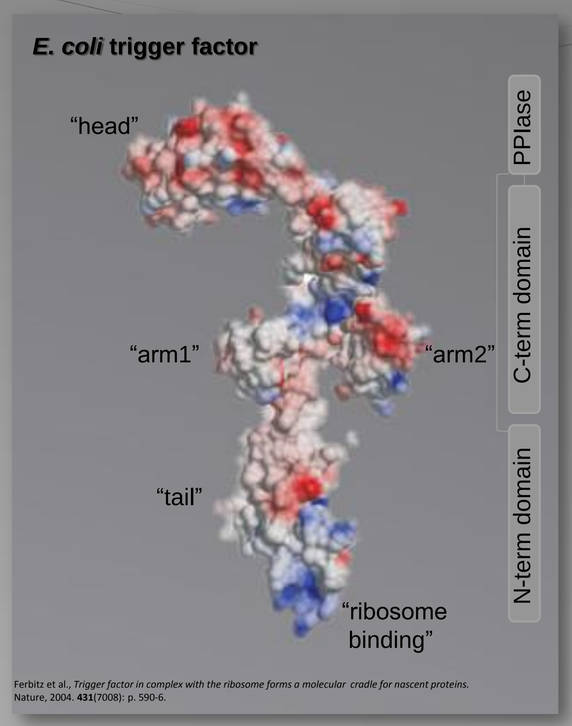
Thermal Adaptation of the Ribosomal Chaperone Trigger Factor

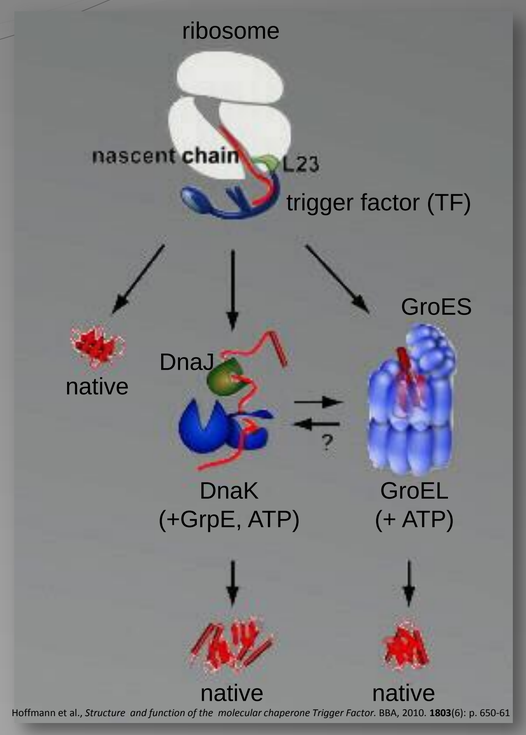
Amandine Godin, Philipp Schmidpeter*, Franz X. Schmid*, Georges Feller
Laboratory of Biochemistry, Centre for Protein Engineering, Institute of Chemistry B6, University of Liège, B-4000 Liège, Belgium
*Laboratorium für Biochemie, Universität Bayreuth, D-95440 Bayreuth, Germany

Introduction

Trigger factor (TF) is a 48 kDa protein involved in folding in bacteria. It is the first molecular chaperone interacting with virtually all newly synthesized polypeptides on the ribosome and also possesses a peptidyl-prolyl *cis-trans* isomerase activity. Proteomics studies have recently revealed that the trigger factor is the main upregulated protein in the psychrophilic microorganism *Pseudoalteromonas haloplanktis* grown at 4°C (compared to 18°C). Moreover, the expression of the two other major bacterial chaperones, DnaK and GroEL, is downregulated at 4°C. Therefore, *ph*TF seems to play a key role in cold adaptation of the Antarctic bacterium.



Ferbitz et al., *Trigger factor in complex with the ribosome forms a molecular cradle for nascent proteins.* Nature, 2004. **431**(7008): p. 590-6.



Hoffmann et al., *Structure and function of the molecular chaperone Trigger Factor.* BBA, 2010. **1803**(6): p. 650-61

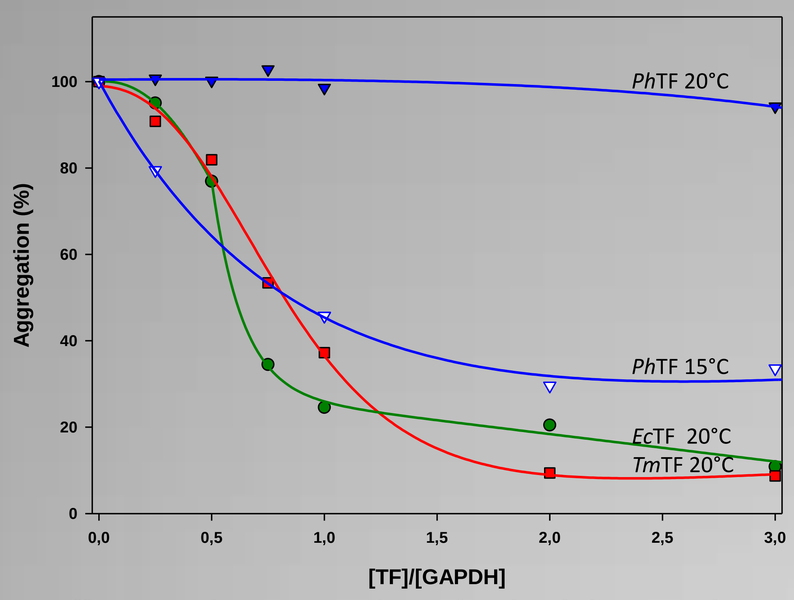
Comparative study of three trigger factors from different thermal origins

Protein	Source	Estimated environmental T°
*Ph*TF	*Pseudoalteromonas haloplanktis* TAC125	< 0°C (psychrophilic)
*Ec*TF	*Escherichia coli* RR1	37°C (mesophilic)
*Tm*TF	*Thermotoga maritima* DSM3109	85-90°C (hyperthermophilic)

In order to study the adaptation of this chaperone to temperature, we have produced and purified three different TFs, and we have investigated their chaperone function, PPIase activity and interaction with unfolded substrates.

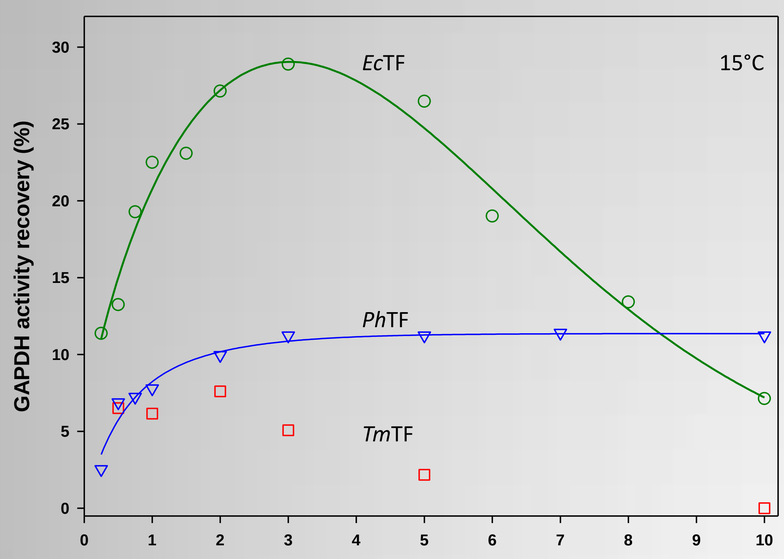
Chaperone activity

Prevention of D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) aggregation by TFs



Effect on the aggregation of GdmCl-denatured GAPDH, induced upon dilution:
***Ec*TF and *Tm*TF -** Gradual prevention of aggregation at 20°C
***Ph*TF** (T_m=33°C) - No protection against aggregation at 20°C, but chaperone activity detected at 15°C after cold incubation

Reactivation of D-glyceraldehyde-3-phosphate dehydrogenase by TFs

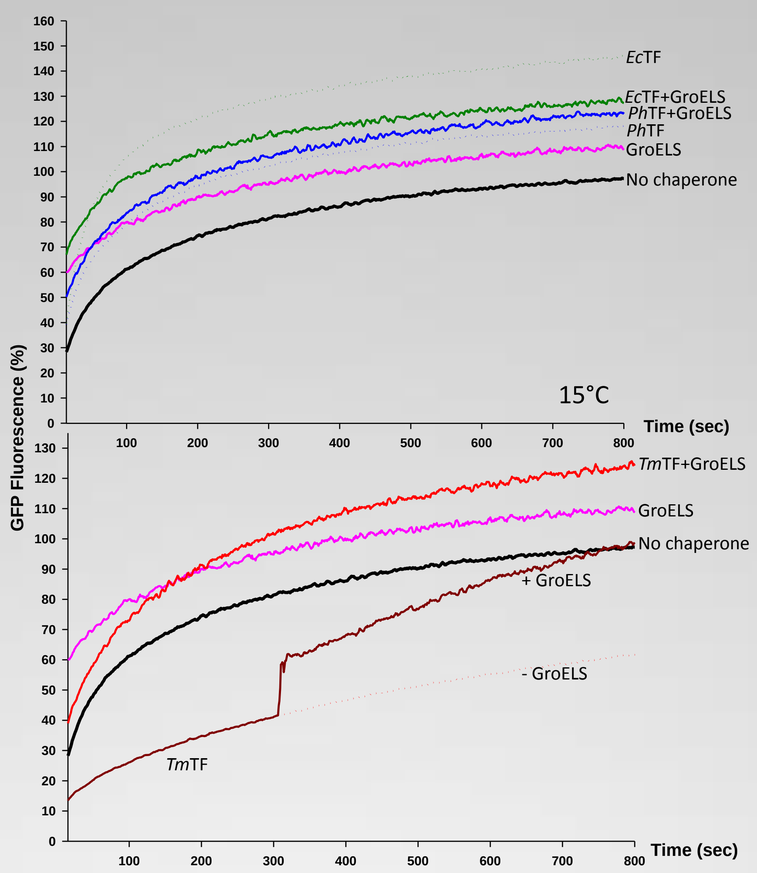


Effect on the reactivation of GdmCl-denatured GAPDH:
***Ph*TF -** Slight improvement
***Ec*TF -** Enhancement at low TF concentration, inhibition at high concentration
***Tm*TF -** Inhibition

Green Fluorescent Protein mutant 2 refolding assay in the presence of TFs

Effect on the refolding of acid-denatured GFP:
***Ph*TF -** Slight improvement
***Ec*TF -** Enhancement at low TF concentration
***Tm*TF -** Inhibition

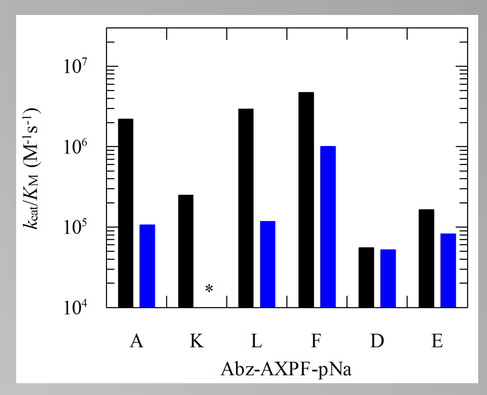
➢ *Consistent with GAPDH reactivation assay*
➢ *Also performed at physiological temperatures of each TFs: results similar to those obtained at 15°C*



Cooperation with the chaperonin GroEL/ES+ATP:
GroELS
- Improvement of GFP refolding yield and acceleration of the first phase of the kinetics
***Ec*TF+GroELS**
- Competition between the 2 chaperones
***Ph*TF+GroELS**
- Slight improvement of the yield
***Tm*TF+GroELS**
- Cooperative effect
- Prior incubation with TmTF is favorable to the refolding

PPIase activity

Tetrapeptide assay



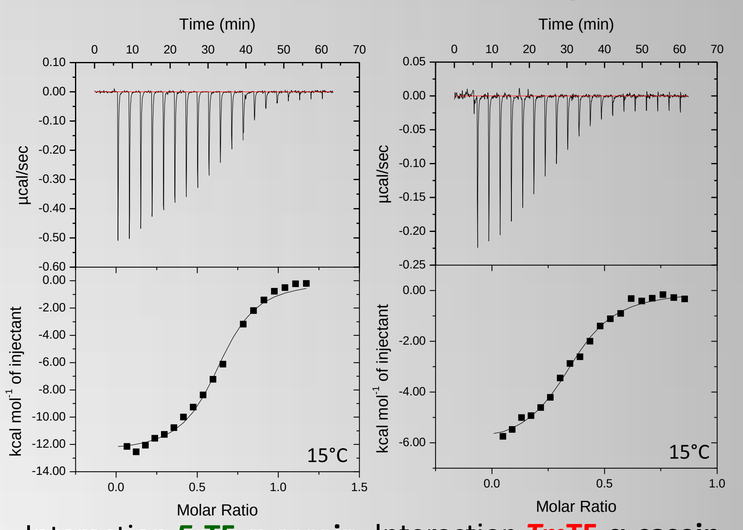
***Ec*TF**
- PPIase activity detected
- Lower specifity towards peptides having a charged residue before P

***Ph*TF**
- PPIase activity detected
- Less active than *Ec*TF, even at 15°C
- Likely similar specifity

***Tm*TF**
- Almost inactive

Interaction TFs-unfolded substrates

Isothermal Titration Calorimetry



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

- ***Ec*TF** and ***Tm*TF**: Binding to an unfolded protein, and not necessary to a refolding protein
- No interaction detected with ***Ph*TF**
- **Stopped-flow affinity kinetics**: high affinity of ***Tm*TF** for RCM-RNase T1

Conclusions

This characterization of extremophilic TFs, performed under identical experimental conditions for the three chaperones, suggests that the trigger factor possesses thermal adaptations related to the strain lifestyle.

➢ **Thermophilic TF** behaves as a holdase, forming a complex with proteins to protect them at high physiological temperatures prone to protein aggregation before their folding by other chaperone systems.

➢ **Psychrophilic TF** promotes the folding at any concentration and acts as a "true" chaperone. The lower efficiency of *Ph*TF may be caused by the experimental conditions which are far from *P. haloplanktis*' life conditions. It is also possible that *Ph*TF acts only during polypeptides synthesis at the ribosome, as very low temperatures prevent aggregation.

➢ **Mesophilic TF** seems to be versatile, with a characteristic holding-chaperone activity.

This fundamental study could have potential applications in biomedical field (diseases due to protein aggregation such as Alzheimer), and for protein expression systems in biotech industry.