Selection for proteins that overcome heat-induced lethality of Δ*degP* strain

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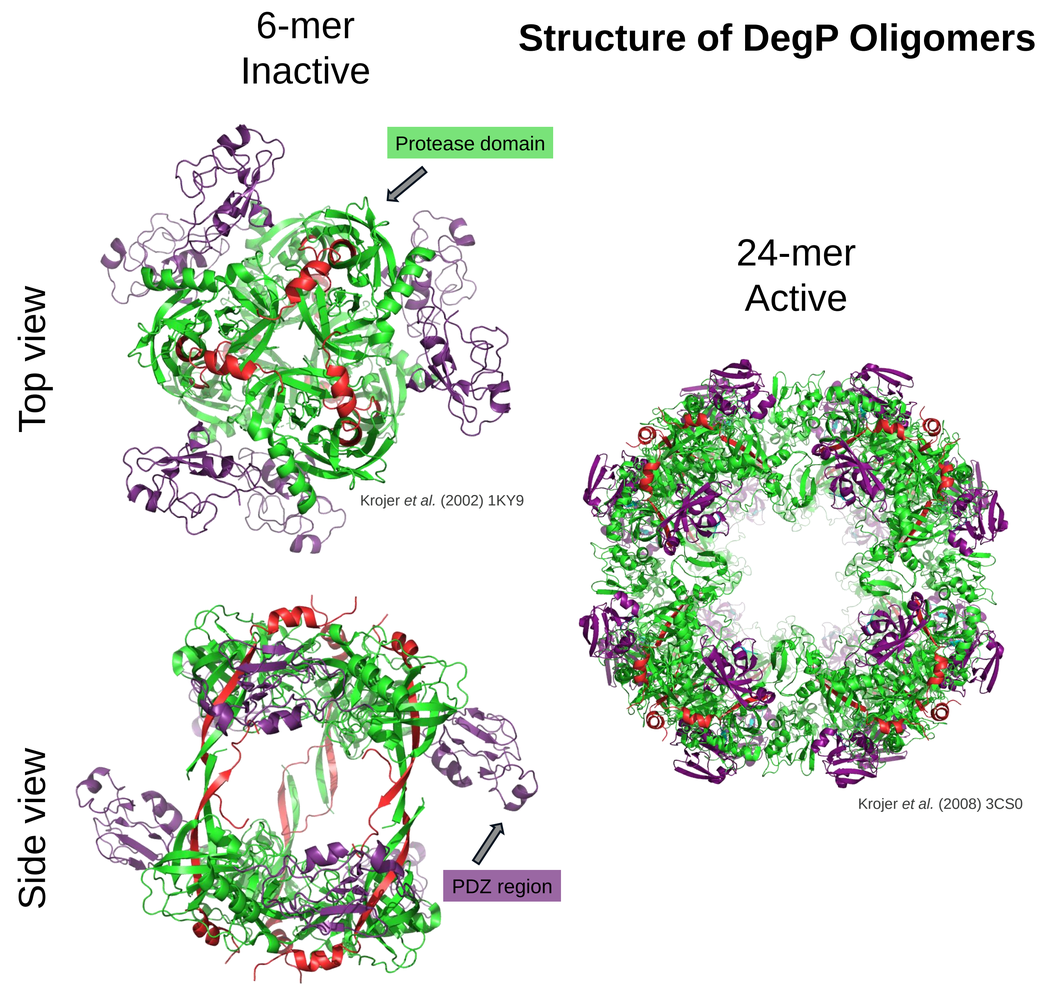
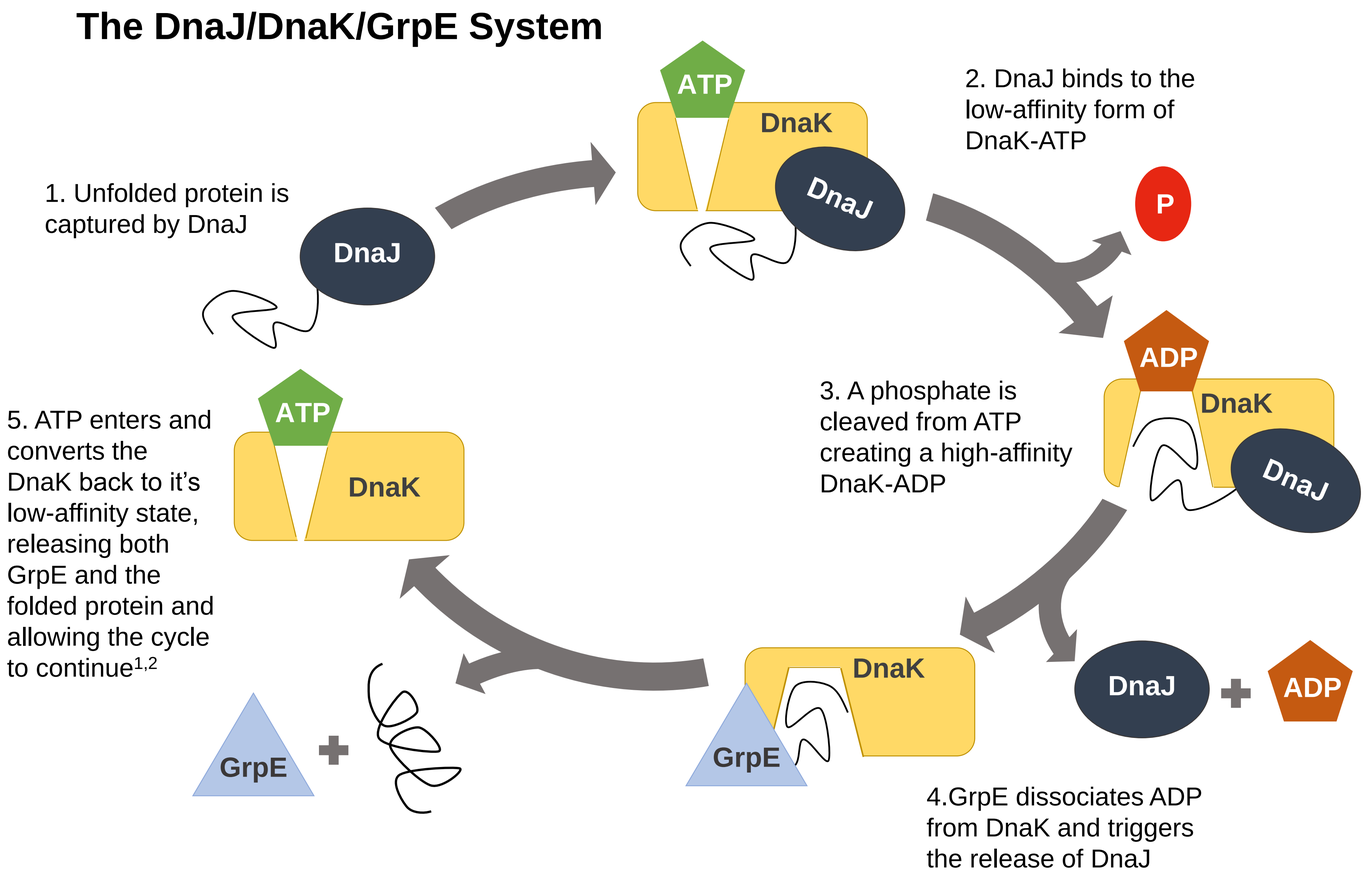
INTRODUCTION

The periplasmic protease/chaperone DegP (HtrA) plays a key role in the quality control of many proteins in the periplasm of *E. coli*. Proteins that fail to fold in the periplasm can be proteolysed, while others are chaperoned to their native folded state by DegP. In a Δ*degP* strain, *E. coli* is unable to survive the protein folding stress at elevated temperatures. Utilizing this phenotype, we developed a plasmid-based selection of suppression of heat-induced lethality in a Δ*degP* strain. Plasmid libraries of various prokaryotic genomes were screened for proteins that overcame heat-induced lethality. Initial hits indicate novel mechanisms of overcoming periplasmic stress, such as the periplasmic expression of a cytoplasmic GrpE homolog and the cytoplasmic expression of an unknown protein.

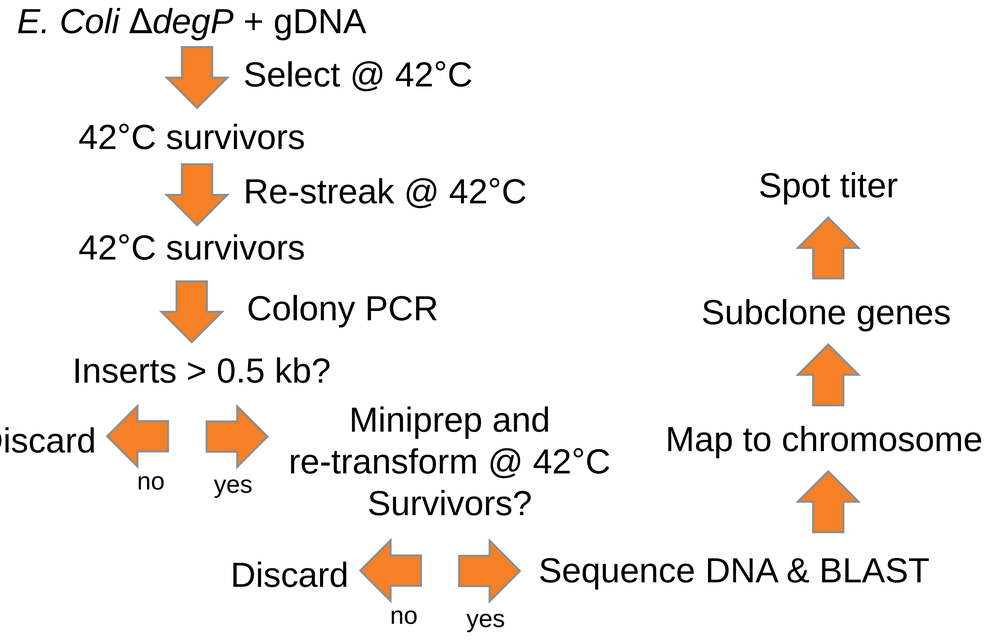
Structure of DegP Oligomers



The DnaJ/DnaK/GrpE System

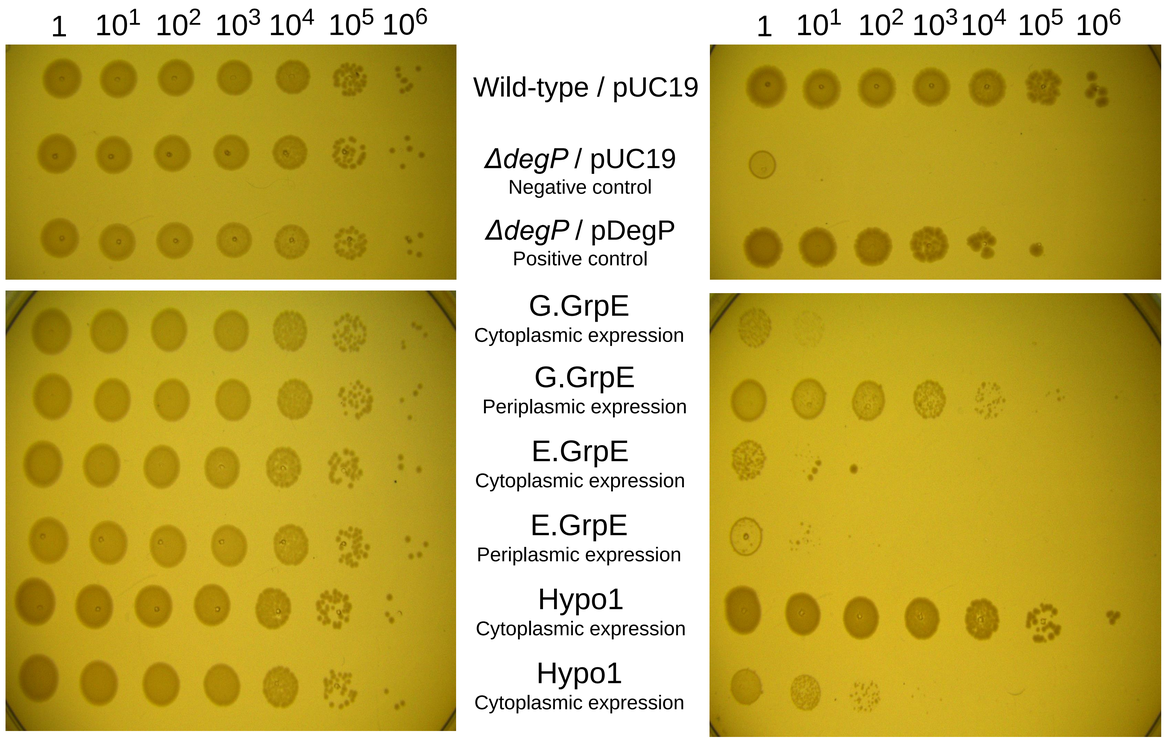


METHODS



RESULTS

Spot titers of confirmed hits



ΔdegP E. coli cells were transformed with cytoplasmic expressing (pMER77) or periplasmic expressing (pMER78) plasmids harboring the genes for *Geobacillus species* GrpE, *E. coli* GrpE, or *Citrobacter amalonaticus* hypothetical protein 1. Serial dilutions of these cells plus the wild type and experimental controls were spotted onto agar containing LB + ampicillin and incubated overnight at either 30°C or 42°C.

Confirmed hits

Gene	Species	Size (bp)	Expressed Compartment	Spot Titer
GrpE	*Geobacillus species*	627	Periplasm/cytoplasm	10^5
Hypothetical transcriptional factor	*Citrobacter amalonaticus*	501	Cytoplasm	10^6
DegS	*Haemophilus influenza*	1020	Periplasm	10^6
Do/hhoA	*Haemophilus influenza*	1392	Periplasm	10^6

FUTURE DIRECTIONS

- Continue Library QC & selection process for all available libraries
- Develop an *in vivo* and *in vitro* protease and chaperone assay
- Use *ΔdnaJ, ΔdnaK,* and *ΔdnaJ ΔdnaK* strains of *E. coli* to identify *Geobacillus* GrpE partners
- Knock-out *E. coli* GrpE in the presence of cytoplasmic *Geobacillus* GrpE
- Determine active site for GrpE in order to knock-out GrpE activity to verify necessity in complementation

REFERENCES

1. Winter, Jeannette & Jakob, Ursula. (2004) Critical reviews in biochemistry and molecular biology. 39. 297-317.
2. Szabo, Alexander *et al.* (1994) The ATP hydrolysis-dependent reaction cycle of the *E. Coli* HSP70 system- DnaK, DnaJ, and GrpE.. Proc. Nadl. Acad. Sci. **91:** 10345-10349.