SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Microbiology

has the honor to invite you to the public defense of the PhD thesis of

Rani Baes

to obtain the degree of Doctor of Bioengineering Sciences

Regulation of heat shock response in the thermophilic Crenarchaeon *Sulfolobus acidocaldarius*

Promotors:
Prof. dr. ir. Eveline Peeters (VUB)
Prof. dr. Wim Vranken (VUB)

The defense will take place on
Thursday, March 9, 2023 at 5 PM
in auditorium D.2.01

Members of the jury:

Prof. dr. Dominique Maes (VUB, chair)
Prof. dr. Charles Van Der Henst (VUB, secretary)
Prof. dr. ir. Geert Angenon (VUB)
Prof. dr. ir. Kiavash Movahedi (VUB)
Prof. dr. Sébastien Ferreira-Cerca (University of Regensburg)
Prof. dr. Louis Droogmans (ULB)

Curriculum vitae

Rani Baes obtained her degree of Master of Science in Biology: Molecular & Cellular Life Sciences at the Vrije Universiteit Brussel in 2018. After graduating, she obtained a FWO fellowship "aspirant fundamental research" and started her PhD in the Research Group of Microbiology in October of 2018.

Rani's work resulted in four international journal articles, four talks and several poster presentations at (inter)national conferences. During her PhD trajectory, she supervised four master thesis students and guided different practical courses for bachelor and master students.

Abstract of the PhD research

Temperature is a crucial environmental parameter for all living organisms. Although it is well understood how bacteria and eukaryotes respond to temperature changes, this is largely enigmatic for archaea. The thermophilic Crenarchaeon *Sulfolobus acidocaldarius* grows optimally at 75°C in volcanic hot springs - habitats that are typified by large temperature gradients. Strikingly, it is unknown how this temperature-sensing and corresponding heat shock response is regulated. This work aims to address these questions by unravelling heat shock response of *S. acidocaldarius* using a system-level perspective and a focused study on the regulation of the major heat shock protein (HSP).

Using a validated heat shock set-up, phenotypical assays determined the condition eliciting maximal heat shock response without affecting cellular viability. At this condition, pulse-labeling of neosynthesized RNA and protein indicated that transcriptional and translational activity was decreased. However, transcriptome and proteome analyses demonstrated an extensive and fast response at the RNA level and a slower reprogramming of the protein landscape. Functional enrichment analysis indicated that nearly all biological processes are affected by heat shock. Our results point towards the absence of a classical transcription factor as the major regulatory mechanism of heat shock response and suggests that transcriptional regulation is established by changes in overall DNA compaction.

A direct correlation between transcriptional and translational expression was not evident for most genes, suggesting the existence of post-transcriptional regulatory processes. One possibility includes regulation by RNA structural elements (RNA thermometers), usually located in the 5' untranslated region (5'UTR). For the HSPs, RNA thermometer structures were predicted *in silico* and validated *in vitro* using an optimized RNA structural probing procedure.

Heat shock responsive regulation was further investigated for the major HSP, by constructing a 5'UTR deletion strain. This 5'UTR region was confirmed as a determinant for correct HSP levels at the optimal growth temperature and for heat-shock responsive upregulation by primer extension, qRT-PCR and western blotting assays. To our knowledge, this is the first demonstration of leader-associated, temperature-responsive regulation in archaea.

With this, my research provides a comprehensive insight into heat shock responsive regulation of a model Crenarchaeon and a better understanding of the physiology of this organism in the context of its natural habitat. Given the unique phylogenetic position of archaea, this research also contributes to a better understanding of the origin and early evolution of temperature-dependent regulation, which is also relevant for the other domains of life.