In vivo envelope stress response of *Salmonella*

P. Maturana[1], A. Schmidt[2], D. Bumann[1]
[1] Infection Biology, Biozentrum, Klingelbergstrasse 50/70, Basel, Switzerland
[2] Proteomics Core Facility, Biozentrum, Klingelbergstrasse 50/70, Basel, Switzerland
E-mail: pauline.maturana@unibas.ch

The *Salmonella* envelope is involved in uptake of nutrients, excretion of waste products, sensing of external stimuli, and secretion of virulence factors. During infection, it is attacked by host effector mechanisms. Envelope stress is sensed by several different pathways, and among these the extracytoplasmic stress response sigma factor E (σ^E, encoded by *rpoE*) seems to be particularly relevant for systemic virulence in mice based on the strongly attenuated phenotype of an *rpoE* mutant (Humphreys *et al.*, IAI 67, 1560). σ^E regulates expression of more than 300 genes including well-known virulence factors such as HtrA. However, it remains unclear which of these genes are particularly relevant for envelope maintenance and what kind of physiological problem they solve.

To address these questions, we generated a transposon library in a *Salmonella enterica* serovar Typhimurium SL1344 *rpoE* strain. We used a transposon that carries two outward facing promoters with different strengths (P_{bla}, P_{tac}) which could activate expression of genes around the transposon insertion site, in addition to disrupting genes hit by insertion. We intravenously infected susceptible BALB/c mice with this library and prepared spleen and liver 5 days post infection. Whereas the parental *rpoE* mutant was completely cleared from infected mice within 5 days, several clones of the library could be isolated demonstrating partial rescue of virulence. Interestingly, one region carrying genes associated with outer membrane porins were hit in different dominating clones. Individual virulence tests revealed that these clones were able to survive for at least 5 days at high levels but did not show net *in vivo* growth. We used proteomics and clean mutations to demonstrate that the lack of one of the major porin OmpC enabled *Salmonella rpoE* to survive in mouse spleen. By contrast, inactivation of another closely related major porin OmpD had no impact suggesting divergent properties for OmpC and OmpD. We are currently investigating the role of various potential differences in *in vivo* abundance and small molecule permeation.

We also performed another round of transposon mutagenesis on the rescued *Salmonella* strain to test if we can further rescue virulence. After two *in vivo* passages, we obtained four different clones that regained considerable *in vivo* growth capabilities. The affected genes are *pnp* which codes for a polynucleotide phosphorylase, *cadA* which codes for a lysine decarboxylase, *ftsI2* which codes for a penicillin-binding protein and *yicH* which codes for an hypothetical exported protein. We are currently further analyzing these clones but the data already suggest that regulation and/or repair of outer membrane permeability determinants might be central to *in vivo* envelope stresses and associated virulence requirements. Together, our results indicate that surprisingly just a few genetic alterations can partially rescue the very strong attenuation caused be a defective major sigma factor with a large regulon.