EnvR is a potent repressor of accrB expression in Salmonella Typhimurium

Pauline Siasat, Helen E. McNeil, Abigail Colclough, Vito Ricci, Amelia J. Lawler, Hind Abdalaal, Michelle M. C. Buckner, Alison Baylay, Stephen J. Busby, Laura J. V. Piddock and Jessica M. A. Blair
School of Medical and Dental Sciences, University of Birmingham, Birmingham, UK

Introduction

Efflux pumps export diverse antimicrobial compounds out of bacterial cells and maintain low internal concentrations. Increased expression of resistance-nodulation-cell division (RND) pumps serve as crucial drivers of multi-drug resistance (MDR) in Gram-negative bacteria1.

Pump expression is negatively regulated by members of the TetR family of transcriptional regulators (TFTRs). Previous work has shown that the TFTR EnvR can repress accrB transcription, the primary RND pump in Escherichia coli. By defining its precise binding residues and phenotypic impact, we show that EnvR can also effectively inhibit the expression of accrAB in Salmonella enterica.

1. A 24-bp palindromic sequence in accrAB promoter is sufficient and required for EnvR binding in S. enterica

Figure 2: Nucleotide substitutions introduced into the accrB promoter. Highlighted in yellow is the predicted 24-bp wild-type binding site of EnvR. Site mutations are in green.

Figure 3: EnvR binds wild-type accrAB promoter but not its mutants. Binding affinity of AccR and EnvR (0.5 mg/mL) to wild-type and mutant (paccrABmut) accrAB promoter (45 ng) determined using an electrophoresis shift mobility assay (EMSA). DNA/protein mixtures separated on 6% native polyacrylamide and stained with SYBR green. Band shifts caused by protein binding to the promoter are indicated by black arrows.

2. Overexpression of EnvR effectively inhibits accrAB expression

Figure 4: Overexpression of EnvR prevented production of accrB mRNA and protein in S. Typhimurium SL1344 envR. (A) mRNA production reduced to a 0.05 fold change compared to SL1344 when EnvR was overexpressed. Real-time RT-PCR data was normalised to 16S expression. (B) AccrR protein production was measured via Western Blot on 12% Bis-Tris gels and detected with anti-AccR primary antibody.

3. Increased susceptibility to antimicrobials and loss of efflux activity following EnvR overexpression

Table 1: Overexpression of EnvR increased the sensitivity of S. Typhimurium SL1344 to antibiotics on a substrate-dependent basis. Minimum inhibitory concentrations (MIC) were determined by broth microdilution as previously described4. Significant reductions in concentration (µg/mL) are in bold.

Figure 5: EnvR overexpression significantly increased accumulation and decreased efflux rates in S. Typhimurium SL1344. Fold change in accumulation of H13342 (A) and efflux rate of I2FDA (B) was determined as previously described4. (n≥3, **p<0.01, ***p<0.0005).

4. EnvR overexpression also affected other phenotypes known to be impacted by efflux

Figure 6: Overproduction of EnvR greatly interfered with the motility and capacity of S. Typhimurium SL1344 to invade human embryonic intestinal cells INT-407. (A) Swimming motility was determined by measuring bacterial growth diameter surrounding the point of inoculation. (B) Adhesion and invasion assays performed as previously described4. (***p<0.0005).

Conclusion

✓ EnvR binds to a 24-bp palindromic sequence upstream of the accrAB operon in S. enterica
✓ EnvR is a potent repressor of accrAB expression that effectively inhibits accrAB mRNA and protein production.
✓ When overexpressed, EnvR can significantly impair AccR-mediated efflux activity, leading to decreased efflux rates, increased susceptibility to antimicrobials, and the inhibition of other phenotypes known to be impacted by efflux in S. Typhimurium SL1344

Future works

Investigate the additional roles that EnvR may play outside of efflux regulation.

Exploiting the functions of regulators like EnvR may be a promising avenue to combat antibiotic resistance.

References