A conserved RNA seed-pairing domain directs small RNA-mediated stress resistance in enterobacteria

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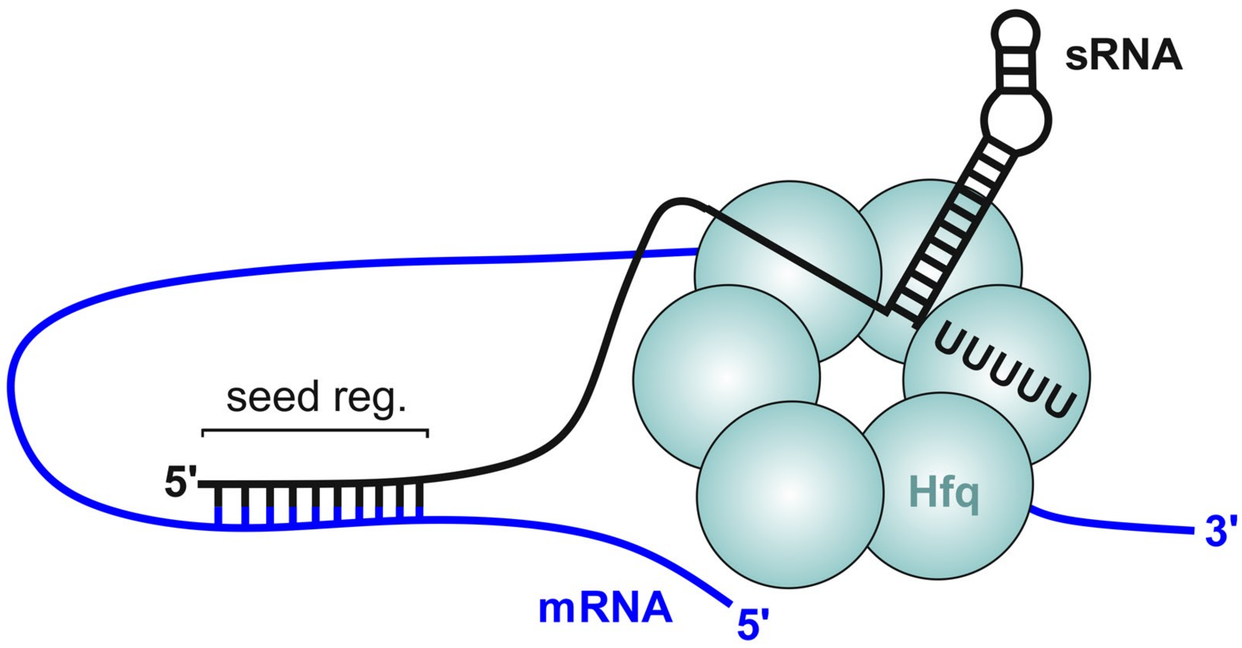
INTRODUCTION

- **Bacterial small regulatory RNAs (sRNAs) are crucial components of many stress response systems.**
- **The envelope stress response is a paradigm for sRNA-mediated stress management and involves the alternative sigma factor E (*rpoE*) and one or more sRNAs.**
- **These sRNAs share conserved functions by regulating outer membrane proteins (OMPs). We speculated that a conserved regulatory motif is responsible for this observation.**
- **In contrast to their protein counterparts, little is known about functional domains present in sRNAs.**

AIMS

- **Test for the presence and relevance of sRNA motifs in sigma E-dependent sRNAs**



METHODS

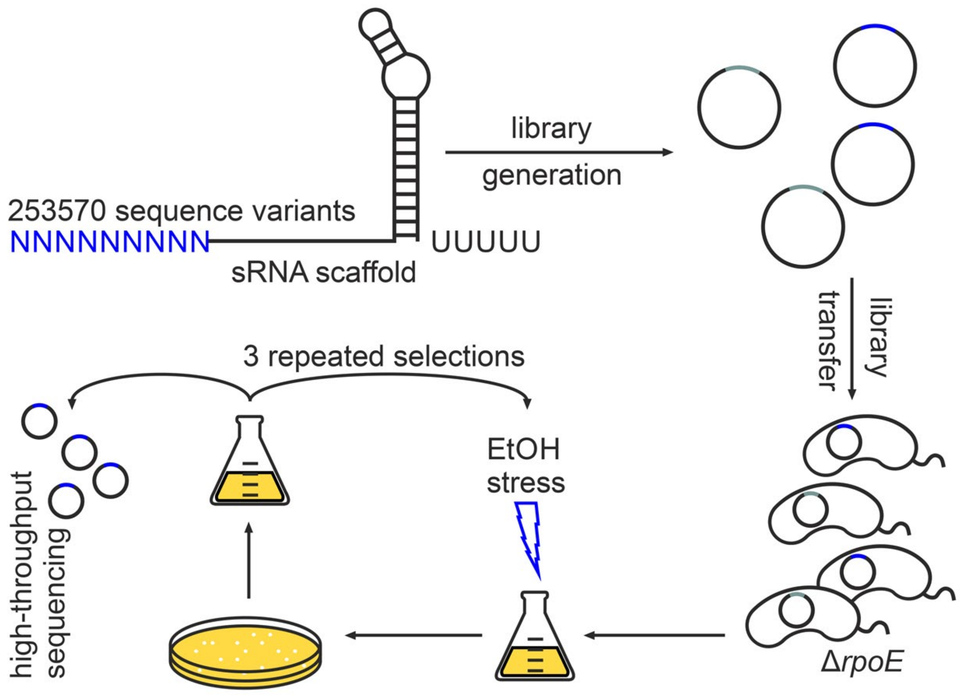


Fig. 1 | Experimental strategy for laboratory selection experiments: an sRNA library was generated using a *rybB* scaffold with nine randomized nucleotides at the 5' end, cloned into plasmid backbones, and transfered in to *V. cholerae* Δ*rpoE* cells. Strains were pooled, treated with ethanol (3.5% final conc.) for 6 hours. Surviving cells were recovered and subjected to consecutive rounds of selection. After each selection, the plasmids of surviving cells were analyzed using high-throughput sequencing.

RESULTS

1

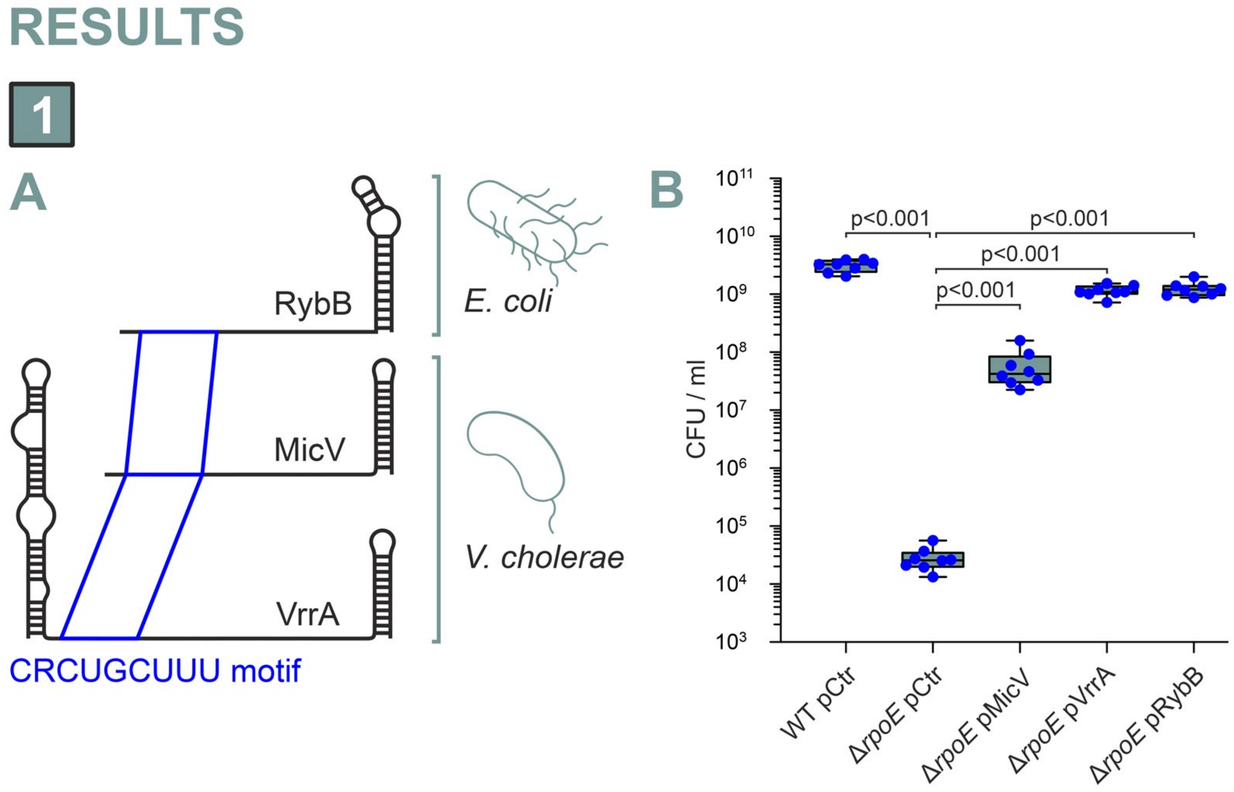


Fig. 2 | **A)** Secondary structures of the sigma E-dependent sRNAs RybB, MicV and VrrA, highlighting the presence of the conserved CRCUGCUUU motif in the seed-pairing regions of the sRNAs.
B) The indicated strains, overexpressing the respective sRNAs, were assayed for survival after ethanol treatment by determining colony forming units (CFU).

2

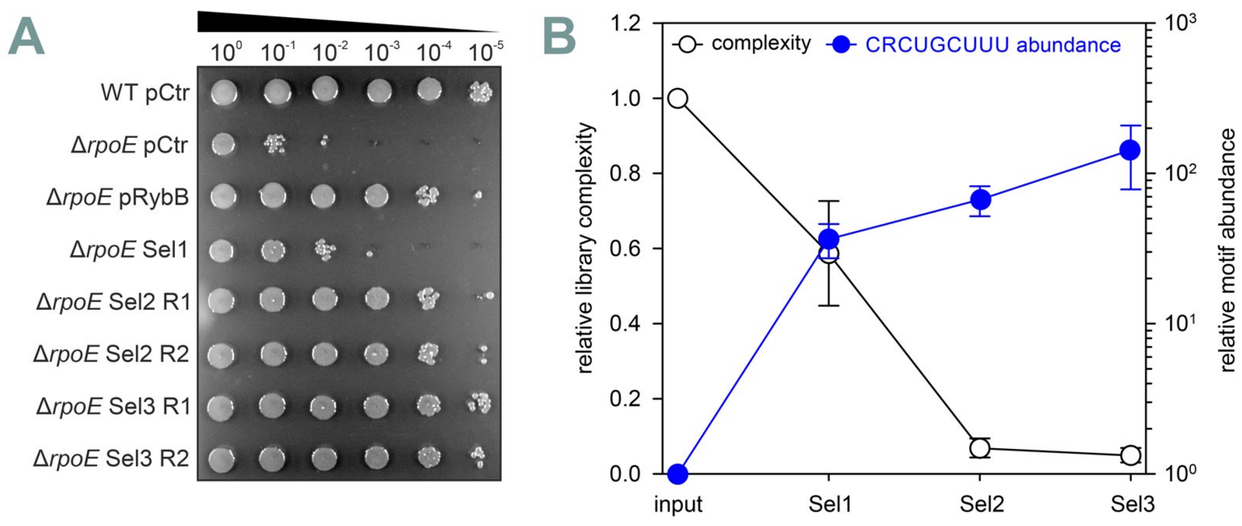


Fig 3 | **A)** *V. cholerae* wild-type and Δ*rpoE* strains carrying control plasmids or the sRNA libraries after consecutive selection rounds (Sel1, Sel2, Sel3) were treated with ethanol and assayed for survival by spotting on agar plates.
B) Plasmid contents of the strains carrying the sRNA libraries were analyzed using high-throughput sequencing. Relative library complexity was determined by counting sequence variants present in the normalized samples. To test for the enrichment of possible sequence motifs, the sequence variants present in each sample were counted and normalized for sequencing depht. The resulting data were analyzed for the enrichment of the conserved CRCUGCUUU motif.

3

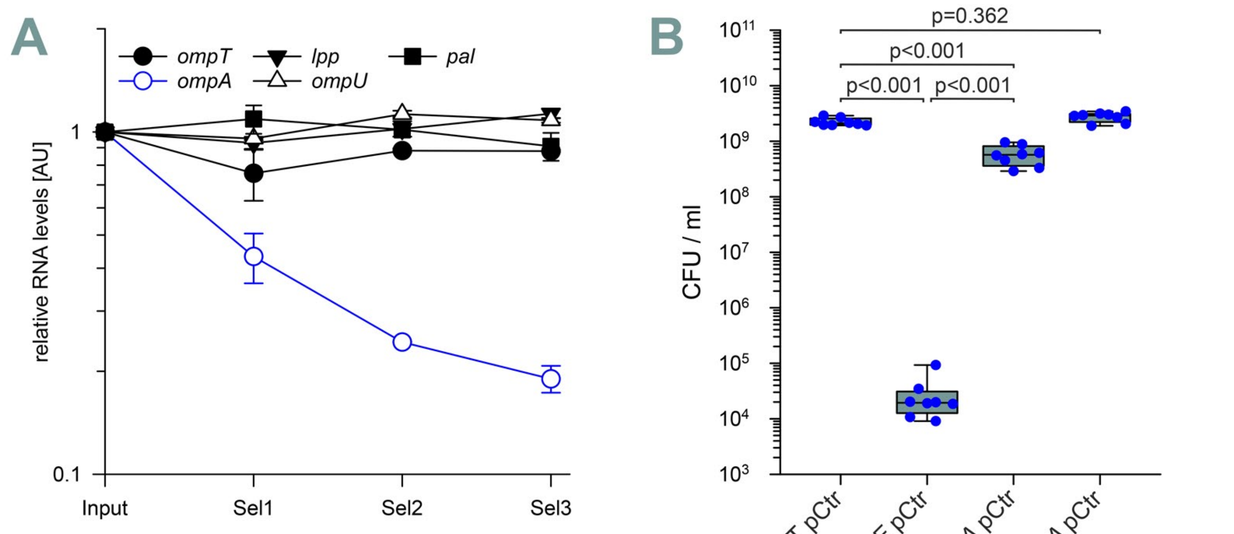


Fig 4 | **A)** *V. cholerae* Δ*rpoE* strains carrying the sRNA libraries before (input) and after consecutive ethanol selection experiments (Sel1, Sel2, Sel3) were assayed for *omp* mRNA levels using qRT-PCR.
B) The indicated strains were assayed for survival after ethanol treatment by determining colony forming units (CFU).

CONCLUSIONS

- **Laboratory selection experiments using synthetic sRNA libraries identified a conserved sRNA motif that is sufficient to suppress ethanol stress sensitivity in *Vibrio cholerae*.**
- **Repression of the major outer membrane protein OmpA is key to sRNA-mediated ethanol resistance.**

REFERENCES

Peschek *et al.*, (In press), 'A conserved RNA seed-pairing domain directs small RNA mediated stress resistance in enterobacteria', *EMBO Journal*

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