OPTIMIZING A BACTERIAL sRNA SCAFFOLD FOR TARGETING MULTIPLE mRNAS, FILTERING OFF–TARGET mRNA INTERACTIONS, AND BALANCING METABOLIC PATHWAY FLUX

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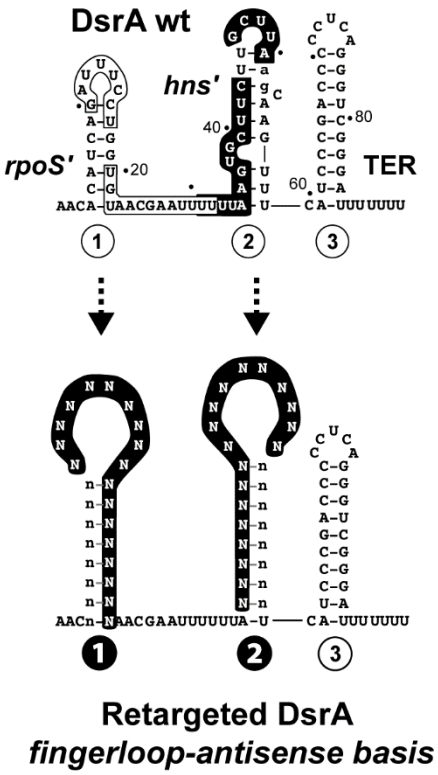
RNA is central to gene expression control in cells, and yet the small regulatory RNAs (sRNAs) of bacteria are still in the early stages of development as synthetic biology tools. The ubiquity and diversity of sRNAs in bacteria bodes well for engineering synthetic sRNA control of metabolic pathways, particularly in organisms with poorly developed genetic tools. These sRNAs regulate mRNA targets by RNA:RNA base-pairing interactions, and sRNAs have been retargeted to regulate non-native mRNAs for metabolic engineering applications.[1-3] However, an ongoing concern about sRNAs as tools is their potential for hybridizing to off-target mRNAs. Here we describe the development, optimization and implementation of a structured sRNA scaffold with improved target discrimination relative to an unstructured antisense sRNA scaffold. Native DsrA sRNA[4,5] contains two striking stem-loop antisense motifs that use antisense base-pairing to coordinately regulate translation of two *E. coli* mRNA targets. Previously[6] we created a genetic system for retargeting DsrA simultaneously to two non-native mRNA targets in *E. coli*. Next, we expressed a retargeted *E. coli* sRNA variant in *C. acetobutylicum* cultures to improve n-butanol biofuel fermentation yield and selectivity. We achieved this goal by retargeting a DsrA sRNA variant to tune-down expression of an essential clostridial hydrogenase and increase NADH levels in the fermentation culture. Finally, we used this *E. coli* sRNA genetic system to demonstrate that the stem-loop antisense "fingerloop" structures can be configured to exclude certain off-target mRNA interactions. This fingerloop antisense motif constitutes a very promising programmable target–mRNA control element that is modular, discriminating, and portable between organisms. Since fine-tuning and balancing metabolic pathway flux is an important scale-up parameter, this nanoscale sRNA tool should be particularly useful in industrial scale bacterial fermentations of biofuels and specialty chemicals.



REFERENCES

(1) Na, D.; Yoo, S. M.; Chung, H.; Park, H.; Park, J. H.; Lee, S. Y. Nat. Biotechnol. 2013, 31, 170.
(2) Liu, Y.; Zhu, Y.; Li, J.; Shin, H. D.; Chen, R. R.; Du, G.; Liu, L.; Chen, J. Metab. Eng. 2014, 23, 42.
(3) Cho, C.; Lee, S. Y. Biotechnol. Bioeng. 2016.
(4) Lease, R. A.; Belfort, M. Mol. Microbiol. 2000, 38, 667.
(5) Lalaouna, D.; Massé, E. Curr. Genet. 2016, 62, 261.
(6) Lahiry, A.; Stimple, S. D.; Wood, D. W.; Lease, R. A. ACS Synthetic Biology 2017, 6, 648.