Investigating mycoplasma small non-coding RNAs using synthetic biology tools

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Abstract

Mycoplasmas are small bacteria that can infect a large range of animals including humans and are characterized by a small genome with a low G+C content. Despite their genomic apparent simplicity, mycoplasmas have to adapt their metabolism and face the immune system of their hosts. In this context, the scarcity of predicted regulators such as alternative sigma factors suggest that small non-coding RNAs may play a central role in the global regulation of gene expression in these organisms. In *Mycoplasma capricolum*, four ncRNAs have been experimentally identified. These ncRNA have no similarity with already characterized bacterial ncRNA and their function remains unknown.
Until recently, any efficient genetic tool was available to precisely delete or modify a gene in mycoplasma. The development of synthetic biology technologies such as genome synthesis, in-yeast engineering and back transplantation into recipient cells have opened up new possibilities to engineer mycoplasma genome.

In order to decipher the function of the ncRNAs detected in *M. capricolum*, a deletion strategy was envisaged on three of them (MCS2, MCS4a, MCS4b). First, the genome of *M. capricolum* was cloned as a centromeric plasmid in yeast after addition of ARSH4-CEN6-HIS3 elements. Deletion of MCS4a, MCS4b and a double deletion was obtained using the tandem repeat coupled with endonuclease cleavage method (TREC). Comparative genomic analyses suggested a functional relationship between MCS2 and the downstream CDS (MCAP0015) that encodes a protein structurally related with H-type ribonucleases. Deletion of MCS2, MCAP0015 and a double deletion were obtained using a CRISPR/Cas9 strategy in combination with homologous recombination driven by 90 nt oligonucleotides pairs used as template. Next step consists in transplanting the modified *M. capricolum* genomes into a suitable recipient cell to get living mycoplasmas with the desired mutations. These mutants will

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then be compared with a non-modified *M. capricolum* in terms of growth and using global transcriptomics and proteomics. In combination with bioinformatics predictions, we aim at identifying the targets of these ncRNA and their biological significance.

Keywords: genome engineering, ncRNA, mycoplasma, Saccharomyces cerevisiae, CRISPR/Cas9, TREC, genome transplantation, seamless gene deletion