Small RNAs, CRISPR, antitoxin antisense RNAs, riboswitches and Hfq as main components of regulatory RNA network in *Clostridium difficile*

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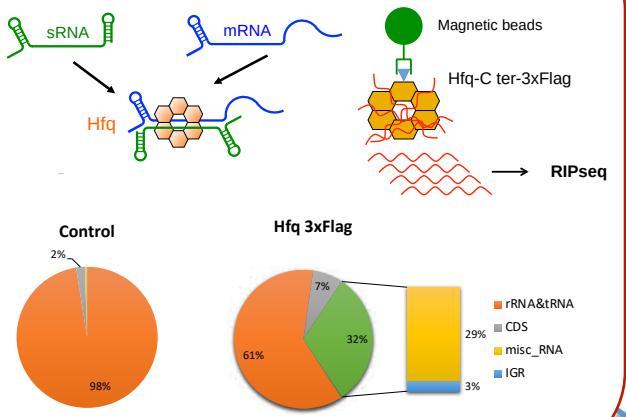
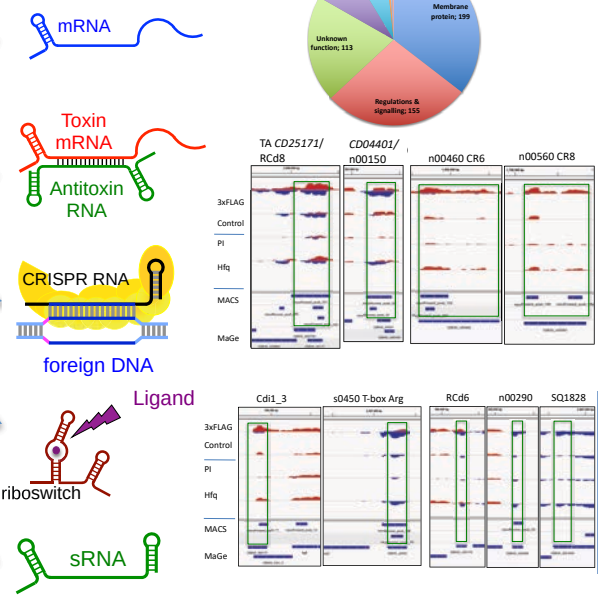
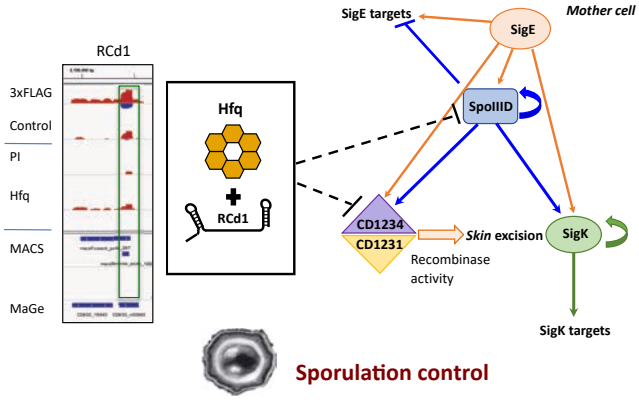
During infection, bacteria reprogram their gene expression in response to environmental constraints. Non-coding RNAs (ncRNAs) play key roles in the regulation of adaptive responses. We are interested in the roles of ncRNAs in the pathophysiology of major human enteropathogen *Clostridium difficile (CD).* We have previously identified a great number (>200) and a large diversity of ncRNAs of different classes in *CD*. These ncRNAs might play important roles in the control of gene expression during the *CD* infection including metabolic adaptations, biofilm formation, stress responses, defence mechanisms and sporulation. A part of these RNAs represents potential *trans*-encoded riboregulators that could require the RNA chaperone protein Hfq for their action. Recent phenotypic and transcriptomic analysis suggested pleiotropic role of Hfq protein in *C. difficile* with the most pronounced effect on sporulation as a key process during infectious cycle for the pathogen dissemination in the environment and persistence inside the host. We performed RNA immunoprecipitation high-throughput sequencing (RIP-Seq) analysis to identify Hfq-associated RNAs in *C. difficile*. Our work revealed a large set of Hfq-interacting ncRNAs and mRNAs, including mRNA leaders and coding regions, known regulatory RNAs and potential new ncRNAs. Among previously identified ncRNAs, in addition to *trans*-encoded RNAs, a number of *cis*-antisense RNAs including antitoxins from all recently identified type I toxin-antitoxin modules, numerous riboswitches and CRISPR RNAs have been enriched in Hfq-associated samples constituting new categories of Hfq ligands. Possible interactions between the identified partners including ncRNA-mRNA and ncRNA-ncRNA pairing were postulated through computational target predictions. Detailed investigation of one of Hfq-associated ncRNAs, RCd1, suggests that this RNA contributes to the control of late stages of sporulation in *C. difficile*. Altogether, these data provide essential molecular basis for further studies on post-transcriptional regulatory network in this emerging enteropathogen.

Hfq RNA immunoprecipitation-sequencing

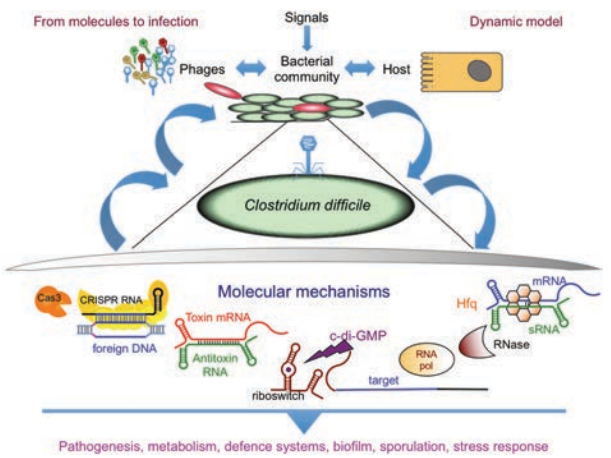




RCd1, Hfq-bound sRNA *in vitro* and *in vivo*



Regulatory RNA network in *C. difficile*



Boudry *et al.* Under revision.