Highlight Team 2 - LuxT controls specific quorum-sensing-regulated behaviors in Vibrionaceae spp. via repression of qrr1, encoding a small regulatory RNA

André Seixas, Bárbara Ferreira, Érika Carvalho, Manuel Carvalho, Maria Carolina Rodrigues

Bacterial communication systems, or quorum-sensing (QS), revealed social ability of microbes, allowing quick responses to fluctuating environments as bioluminescence activation. QS bacteria are self-made, as they produce, release and sense signal molecules called the autoinducers (AI). *Vibrio harveyi* is a model marine bacterium that applies QS in the control of over 600 genes. In this specie five non-coding small regulatory RNAs (sRNAs), Qrr1-5, are responsible for the core of the signalling pathways. At low cellular density (LCD), Qrr sRNAs regulate quorum-sensing. The opposite signalling occurs at high cellular densities. Previous studies pointed a possible differential regulation of *qrr* genes based on variation in promoter regions, and different transcripts concentrations. The aim of this work is to elucidate whether other regulators (Lux family) are involved in *qrr* control *in vivo.*

Electrophoretic mobility shift assays (EMSA) confirmed that LuxT binds upstream of *luxO,* accordingly with previous data. It was shown that LuxT absence (*ΔluxT*) decreases light production in a cell density dependent manner. Moreover, regarding transcription, LuxT does not repress *luxO*, but in contrast influences *luxC* in LCD. These results show that LuxT binds upstream *luxO* but does not repress it, being able to promote QS through an alternative mechanism.

Given the proximity of *qrr1* promoter to the previously identified LuxT binding region the authors propose that LuxT binding will repress *qrr1* transcription. Results showed that *qrr1* expression was increased in *ΔluxT*, contrarily to *luxO*. This regulation of *qrr1* via LuxT is specific to only Qrr1 sRNA.

To test a possible dual regulation mechanism by LuxT on Qrr1 target genes, they evaluated the expression of four target genes *in V.harveyi* strains without *qrr 1-5*. It was found that *ΔluxT* leads to a reduction in the RNA levels of the target genes. These results revealed that during transcription, LuxT regulates Qrr1 target genes in a Qrr1-independent manner.

Next, the authors evaluated post-transcriptional regulation of these target genes via LuxT and Qrr1. Strains without qrr1 have a higher expression of the respective target genes, which suggests a control in a Qrr1-dependent manner. Additionally, haemolytic aerolysin (Qrr1 target gene) activity was quantified, showing that Qrr1 absence leads to a higher activity. The double mutant (Δqrr1 *ΔluxT*) lacks haemolytic activity demonstrating that transcriptional effect of LuxT overrides the post-transcriptional effect of Qrr1.

Finally, the authors evaluated whether the regulation of Qrr1 regulation by LuxT was conserved between species, concluding that this mechanism was also observed in *Aliivibrio fischeri*.

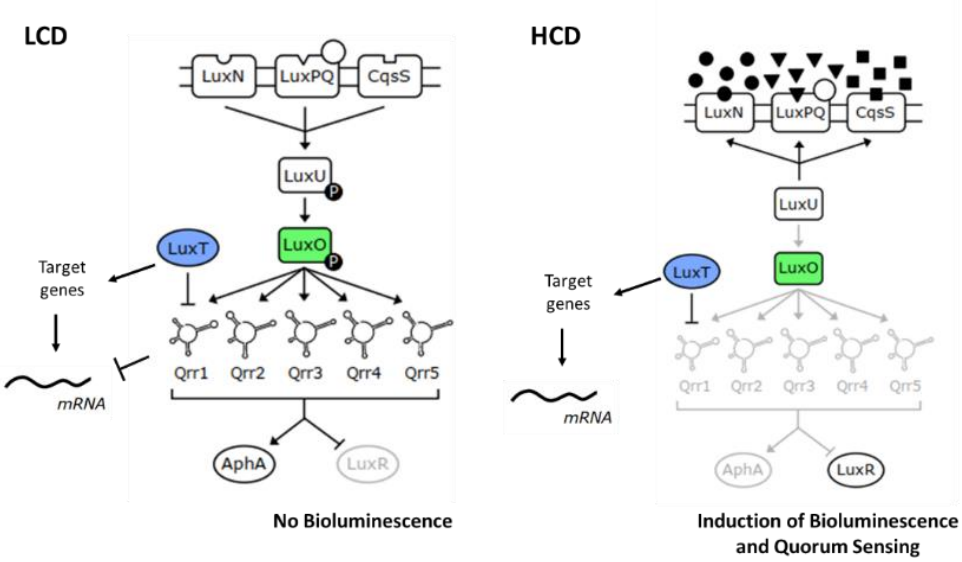

Fig.1. The findings of this work support a new QS model for *Vibrio harveyi*, with the incorporation of a LuxT-mediated repression of one of the core signalling genes *qrr1*.