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An Hfq-binding sRNA in *Listeria monocytogenes* regulates a virulence adhesin in an Hfq-independent manner

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**Introduction**

The small non-coding RNA *LhrC* is conserved among all *Listeria* species, was shown to bind to the RNA-binding protein Hfq, and is present in five sequentially almost identical copies which vary from 111 to 114 nt in size (Christiansen et al. 2006).

In 2009 *LhrC* was found to be highly expressed in blood (Toledo-Ortiz et al. 2009), and recently, Mtcheil et al. (2011) demonstrated that the sRNA is also expressed when *L. monocytogenes* resides within a macrophage cell. It can therefore be assumed that *LhrC* is very important for the pathogen when dealing with the harsh conditions within a host and thus relevant for a successful infection from the pathogen point of view.

**LhrC** is induced during cell surface stress

*LhrC* is induced by a whole range of cell surface acting agents (cefuroxime, bile salts, ethanol, etc.) as seen from Northern Blot analysis (right). Notably, there is no *LhrC* signal in cells lacking the response regulator of the two-component-system *LisRK*, indicating an imperative of *LisRK* for *LhrC* expression.

Growth experiments revealed a growth defect of *LisRK* indicating an imperative of *LisRK* for *LhrC* expression.

All five *lhrC* promoters are active

In order to determine the promoter activity of the single *lhrC* copies each of the five promoters was transcriptionally fused to the reporter gene *lacZ*. The activity of all five promoters increases dramatically after cell surface stress as shown for cefuroxime stress.

Promoters *lhrC1* and the sequentially encoded *lhrC* promoter are being strongest induced after cell surface stress.

However, induction of all five promoters is entirely lost in a *lhrC* mutant background.

Summary

- *LhrC* is induced and important for growth during cell surface stress
- *LisRK* is mandatory for *LhrC* expression
- All five *lhrC* promoters are active with *lhrC1* and *lhrC5* being most active
- *lapB* mRNA is stabilized and translated at a higher rate in *ΔlhrC1-5* after cefuroxime stress indicating a direct interaction between *LhrC* and *lapB*
- *LhrC* binds Hfq, but its interaction to *lapB* is not enhanced by the protein

LapB (*lmo1666*) - direct target of *LhrC*

The top hits of a bioinformatics search for putative targets of *LhrC* (RNApredator, Eggenhofer et al. 2011) were analyzed via RT-qPCR comparing mRNA levels of WT and *ΔlhrC1-5* after cefuroxime stress. The most pronounced difference was obtained for *lapB* mRNA (upper figure) encoding a cell wall protein recently identified as a virulence determinant (Bres et al. 2010).

According to RNApredator *LhrC* binds to the ribosome binding site (RBS) of *lapB* indicating that *LhrC* mRNA is involved in target recognition and the translational fusion of *lapB* to *lacZ* revealed a more than three-fold higher expression in *ΔlhrC1-5* compared to WT after cefuroxime stress (lower figure).

Hfq does not facilitate binding of *LhrC* to *lapB*

Binding of *LhrC* to *lapB* is shown in vitro in gel shift experiments. Even though *LhrC* binds to Hfq, the protein does not enhance the interaction of the two RNAs (upper figure). The investigated sequence of the *lapB* RNA does not bind Hfq and appears in two bands in the gel, both of which are shifted with increasing *LhrC* concentration (lower figure).

Perspectives

- *In vivo* experiments are currently undertaken to substantiate the direct interaction of *LhrC* and *lapB*
- *LapB* upregulation in *ΔlhrC1-5* will be demonstrated on protein level
- Global transcriptomics and proteomics techniques will be used to further unravel the regulatory role of the sRNA

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