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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-Arana et al. 2009), and recently Maheil 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 Δ*lhrC*-5 in presence of 0.07% bile salts (left) and 4 µg/ml cefuroxime (not shown).

**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.

**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 Δ*lhrC*-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 Δ*lhrC*-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 (Ren et al. 2010). According to RNApredator LhrC binds to the ribosome binding site (RBS) of lapB encoding a cell wall protein recently identified as a virulence determinant (Ren et al. 2010). According to RNApredator LhrC binds to the ribosome binding site (RBS) of lapB blocking translation. A translational fusion of lapB to lacZ revealed a more than three-fold higher expression in Δ*lhrC*-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 them 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 Δ*lhrC*-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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