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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-Arama et al. 2009), and recently, Mraheil 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 ΔlhrC1-5 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.

Promoters lhrC1 and the secondarily encoded lhrC5 promoter are being strongest induced after cell surface stress.

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

**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 RNA (upper figure). The investigated sequence of lapB RNA does not bind Hfq and appears in two bands (ΔlhrC1-5 compared to WT after cefuroxime stress lower figure).

**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

**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