Five homologous small RNAs are involved in the response of Listeria monocytogenes to cell wall acting antibiotics

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Five homologous small RNAs are involved in the response of *Listeria monocytogenes* to cell wall acting antibiotics

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**Introduction**

*Listeria monocytogenes* is a food borne pathogen which has the ability to survive and adapt to a wide range of extreme environments; e.g. low pH, low and high temperatures, high osmolarity and the presence of antibiotics. Small RNAs have been found to play a crucial role in stress adaption. Small RNAs exist in a multitude of organisms as stable RNA transcripts which range from 20-500 nt in size, and are typically encoded from intergenic regions. Regulation by small RNAs is mainly achieved through an antisense mechanism at the post-transcriptional level.

In *L. monocytogenes* more than 50 small RNAs have been identified so far, but the elucidation of their role is still in progress (3). The *Listeria* Hfq-binding RNA C (LhrC) was originally found by co-immunoprecipitation with the RNA chaperone protein Hfq (1), which in both gram-positive and gram-negative bacteria, has been shown to enhance the antisense regulation of small RNAs (2,4). By computational approaches it was found that *L. monocytogenes* contains five homologous copies of LhrC. LhrC1-4 are encoded from the intergenic region between cyaK and rld, whereas the fifth copy, LhrC5, is encoded from the intergenic region between bmo0946 and bmo0947 (fig. 1a). Alignment of all five *lhrC* copies shows a great variance among the promoter regions of *lhrC1-5*, whereas the coding regions of *lhrC1-5* are conserved (fig. 1b).

**LhrC1-5 are induced in response to cell wall stress**

To understand the regulatory role of LhrC1-5, transcriptional fusions of the promoter region of *lhrC1, lhrC4* and *lhrC5* to lacZ were constructed and introduced into *L. monocytogenes* EGD-e. Beta-galactosidase assays were conducted to point out which stress conditions *lhrC1-5* may respond to.

The promoter activity was examined in response to a random selection of environmental stress agents. Samples were extracted at after 0, 30 and 60 minutes of stress, respectively (fig. 2a-2c).

All five small RNAs are induced in response to the presence of cell wall stress agents such as ethanol and cell wall acting antibiotics.

**LhrC1-5 are fully dependent on the two component system LisRK**

To analyze the promoter activity and transcription of LhrC1-5 in mid-exponential growing cells upon the addition of ethanol and beta-lactam antibiotics, we conducted beta-galactosidase assays (2% ethanol, 4 µg/ml Cefuroxime and 0.1 µg/ml Penicillin G) and quantitative real-time PCR (4 µg/ml Cefuroxime) experiments. The oligos used for qPCR were designed to recognize all five copies of *LhrC*. Results from fig. 2a-c indicate that LhrC1-5 are induced in response to conditions that activate the two-component systems LisRK and CesRK. Mutants lacking LisR and CesR were therefore included in these assays. The expression of *LhrC1-5* is fully dependent on the presence of transcription factor LisR (fig. 3a and 3b). The response regulator CesR adds another layer of complexity to the regulation of LhrC1-5 expression (fig. 3a).

**LhrC 1-5 stability does not depend of the Hfq chaperone**

To examine whether the chaperone Hfq affects the stability of LhrC1-5, a stability assay was performed, including a wildtype EGD-e strain and a mutant lacking Hfq. LhrC1-5 were induced by the addition of 0.1 µg/ml penicillin G. The RNA turnover was followed after the addition of rifampicin at time 0 minute (which equals 30 minutes induction by pen G). Samples were extracted at time -2, +2, +4, +8, +16 and +24 min relative to addition of rifampicin.

By northern blotting using a probe recognizing all five small RNAs, it is observed that Hfq does not affect the stability of LhrC1-5 (fig. 4).

**Perspectives**

To summarize:

- All five LhrC1-5 were originally identified as Hfq-binding small RNAs (2).
- Alignment of all five copies of *lhrC* shows a great variance among the promoter regions of *lhrC1-5* (fig. 2a-2c).
- All five small RNAs are induced in response to the presence of cell wall stress agents (fig. 2a-c).
- The expression of *lhrC1-5* is fully dependent on the presence of transcription factor LisR. Response regulator CesR adds another layer of complexity to the regulation *lhrC1-5* expression (fig. 3a).
- Hfq does not affect the stability of *lhrC1-5* (fig. 4).

The gene regulation downstream of LhrC1-5 is currently being pursued; do they act as antisense RNAs in an additive, redundant or hierarchical fashion, or by combination of these mechanisms? The answer may lead to an understanding of how *L. monocytogenes* endure the presence of cell wall acting agents in the environment.

**References**


