Bacterial non-coding small RNAs: modes of action and regulatory circuitry

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SalsARN (Programme Blanc 2007)

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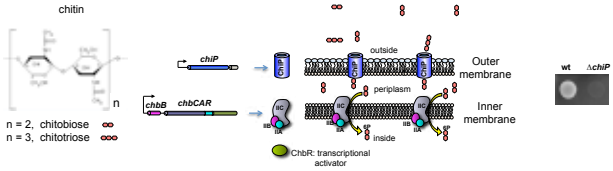
- Small non-coding RNAs have emerged as important components of the regulatory repertoires of all living cells.

- In gram-negative bacteria, a relevant group of small RNAs (sRNAs) acts in concert with chaperon protein Hfq to modulate translation or stimulate degradation of target mRNAs.

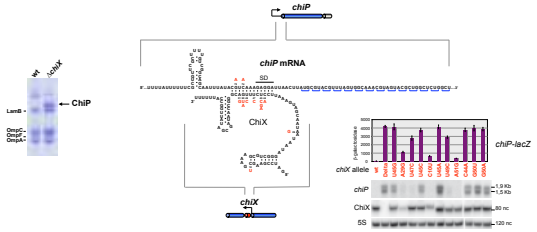
- The small size and the ability to act post-transcriptionally allow sRNAs to provide rapid regulatory responses to environmental changes or stress conditions. Common targets of sRNA regulation are outer membrane porins, whose primary function is to mediate the exchanges with the environment. Two such systems, characterized in the framework of the "SalsARN" project are described below.

"L'arroseur arrosé": Regulatory sRNA inactivated by an inducible transcript mimicking its target.

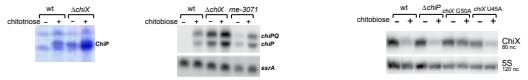
Gram-negative bacteria like *Escherichia coli* and *Salmonella* can use the breakdown products of chitin as sole source of carbon and nitrogen. This metabolic pathway begins with: *i*) the diffusion of the chito-oligosaccharides through a dedicated porin (ChiP); *ii*) their active transport from the periplasm across the inner membrane through a PEP-dependent phosphotransferase system (the ChbBCA complex, encoded by the *chb* operon).



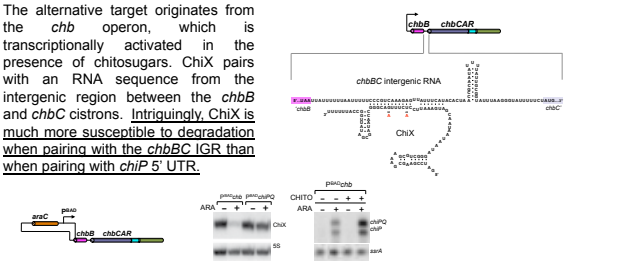
Under most growth conditions, the *chiP* gene is kept silent by a constitutively made sRNA, ChiX, which base pairs with a sequence in the 5' UTR of *chiP* mRNA. Pairing inhibits translation and stimulates degradation of chiP mRNA. Mutations that disrupt pairing or destabilize ChiX cause gratuitous *chiP* induction.



Presence of chito-oligosaccharides in growth medium relieves ChiX repression allowing ChiP protein to accumulate in the outer membrane. Relief of repression is due to the degradation of ChiX sRNA. We found that ChiX is degraded as a result of pairing to an alternative mRNA target.



The alternative target originates from the *chb* operon, which is transcriptionally activated in the presence of chitosugars. ChiX pairs with an RNA sequence from the intergenic region between the *chbB* and *chbC* cistrons. Intriguingly, ChiX is much more susceptible to degradation when pairing with the *chbBC* IGR than when pairing with *chiP* 5' UTR.



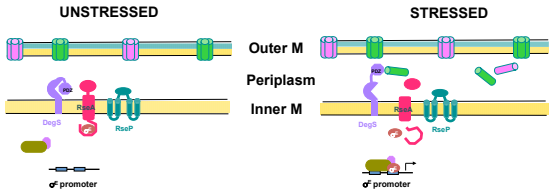
This unique regulatory system effectively couples the passage of chitosugars across the outer membrane with their active transport across the inner membrane.

The structural features and enzymatic activities underlying the *chbBC*-mediated degradation of ChiX are under study.

Figueroa-Bossi, N. et al. *(2009)* ***Genes & Dev 23:*** *2004-2015.*

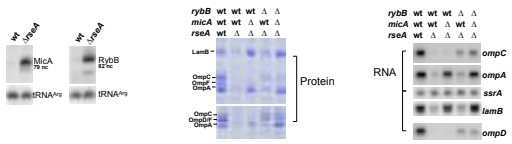
Regulatory sRNAs and envelope homeostasis.

Activation of alternative sigma factor σ^E serves as bacterial paradigm for regulated intermembrane proteolysis (RIP). During unchallenged exponential growth, σ^E is sequestered on the cytoplasmic face of the inner membrane by the binding of transmembrane anti sigma factor RseA. Under stress conditions – including stationary growth – accumulation of unfolded or misfolded OMP polypeptides in the periplasm triggers a proteolytic cascade (involving DegS and RseP proteases) that ultimately destroys RseA and frees σ^E. The latter associates with RNA polymerase core enzyme and transcribes a set of genes whose products (periplasmic folding catalysts, chaperons and proteases) restore folding or degrade defective OMPs.

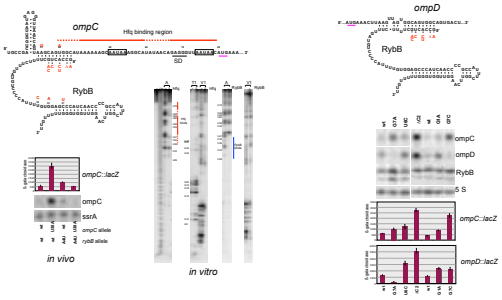


We (and others) have found that the σ^E regulon includes the genes for two regulatory sRNAs: MicA and RybB. Significantly, these two sRNAs target major OMPs' mRNAs: MicA represses OmpA and LamB; RybB represses several OMPs including OmpC, OmpD (see figure below).

In repressing OMP synthesis, MicA and RybB turn off σ^E activation and thus, they turn off their own transcription. Thus, the two sRNAs are at the center of a homeostatic loop.



We have characterized the RybB recognition sequences in *ompC* and *ompD* mRNAs *in vitro* (by structural probing) and *in vivo* (by mutational analysis). These sequences are at entirely different locations: 40 nucleotides upstream from the initiating AUG in the case of *ompC*; within the first portion of the coding sequence in the case of *ompD*.



A sequence as short as 7 nucleotides is necessary and sufficient to confer susceptibilty to RybB regulation.

Balbontín et al. *(2010)* ***Mol Microbiol 78:*** *380-394.*
Balbontín et al. *(2008)* ***J. Bacteriol 190:*** *4075-4078.*
Bossi et al. *(2008)* ***Biochimie 90:*** *1539-1544.*

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