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Design of synthetic riboswitches to regulate RNA stability

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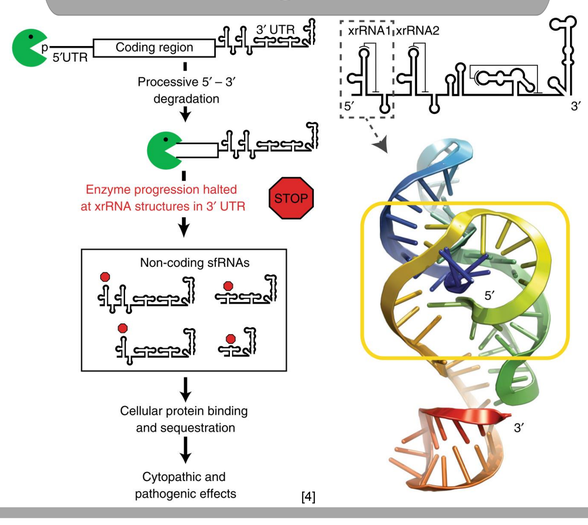
Background

After cell entry, viruses have to overcome many mechanisms that protect the host from infections. *Flaviviruses* like the Zika or Dengue virus developed a strategy to avoid the degradation of viral RNA by the host and also mediate their cytotoxicity and pathogenesis.[1] Their RNA genome forms a structure in the 3'-UTR that is called exoribonuclease-resistant RNA (xrRNA). Acting as a road block, these structures stall the 5'-3'-exoribonuclease Xrn1 so that the downstream located RNA region is not degraded.[2]

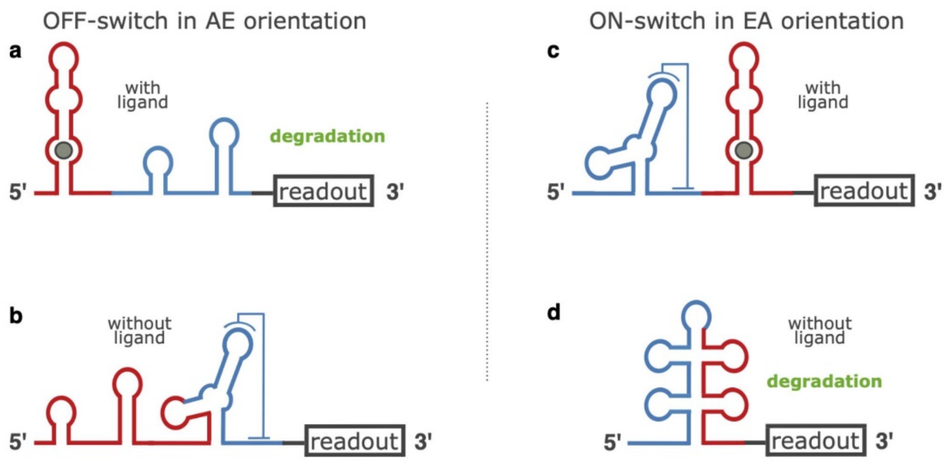


[4]

The mechanical block of Xrn1 is caused by a ring-like RNA structure, where the 5'-end of the RNA is threading through.[3,4] The xrRNA structure is not defined by a distinct sequence, and the Xrn1 stalling is not caused by specific interactions between RNA and exonuclease. Hence, xrRNA is also resistant against other 5'-3'-exoribonuclease like RNase J1 or Dxo1.[4] Important features of the xrRNA structure are a three-way junction and at least one pseudoknot, which creates and stabilizes the ring-like shape.[5] This mechanism of exonuclease stalling is not limited to flaviviruses, but is also shown in plant virus RNA.[6]

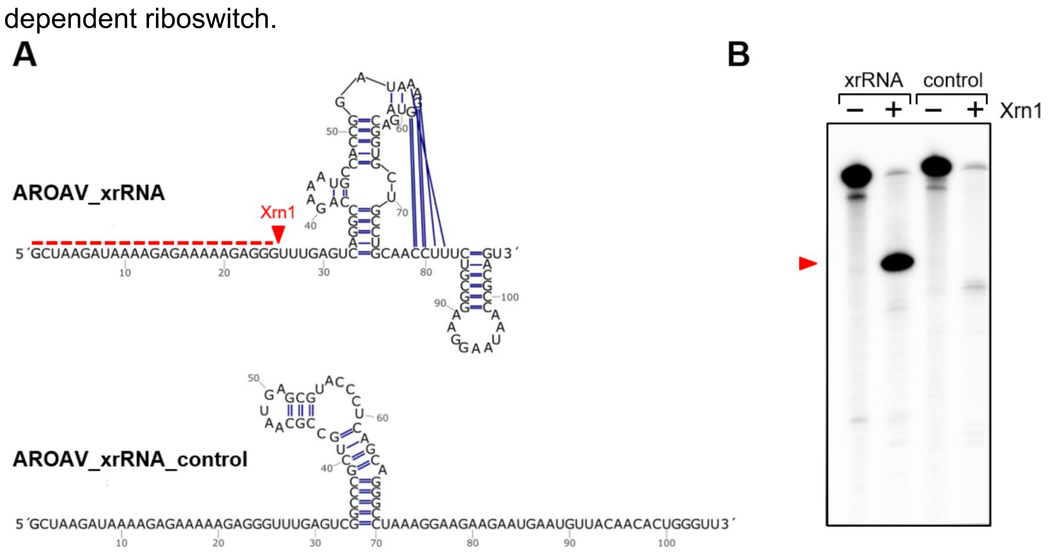
Concept

Riboswitches are regulatory elements that are mostly found in the 5'-UTR of mRNA in bacteria. They have a modular structure consisting of a sensor and an expression platform, making it easy to design synthetic riboswitches. In our project, we use a rational approach to develop novel riboswitches consisting of a theophylline aptamer as a sensor and an xrRNA structure to control the half-live of downstream located mRNA or lncRNA through a ligand-inducible conformational change. The modularity of riboswitches and properties of xrRNA allow for the design of ON- and OFF-Switches with different platform orientations.



E: expression platform; A: aptamer platform

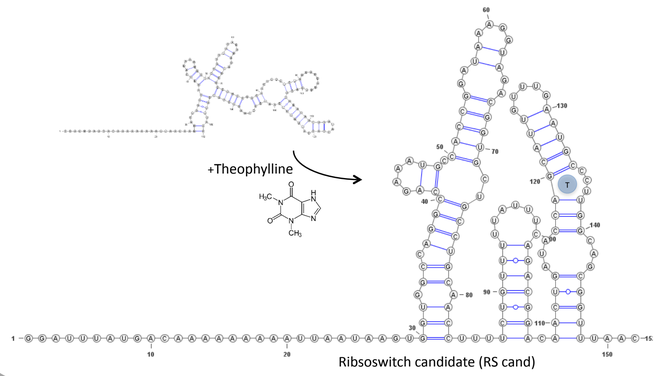
In a pilot experiment, the xrRNA from the mosquito-borne Aroa virus was tested for its resistance against Xrn1. We were able to show the halt of Xrn1 upstream of the xrRNA element, allowing us to use this structure as an expression platform in a theophylline-dependent riboswitch.



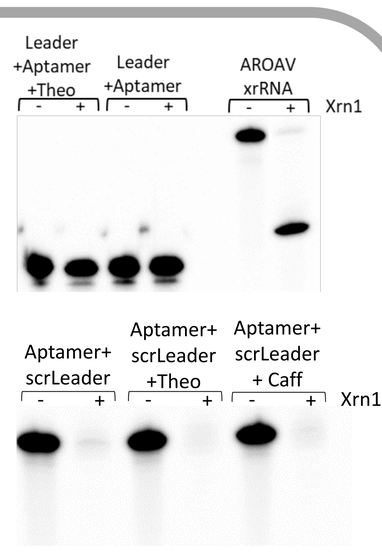
The *in vitro* transcripts of the designed riboswitch candidates are tested for their ability to block Xrn1-mediated degradation, and their secondary structures are identified by in-line probing. The results are then used to improve the rational riboswitch design and to adapt the xrRNA riboswitches for *in vivo* testing in *Saccharomyces cerevisiae*.

Investigations

The synthetic riboswitch candidates (RS cand) as well as their individual components are investigated by in-line probing and tested for stability against Xrn1-mediated degradation.



When theophylline (Theo) was added, the presented riboswitch candidate was protected from degradation by Xrn1, including the leader region. Testing the control constructs showed that the xrRNA structure itself was not protected from degradation. Interestingly, the leader seems to interact with the aptamer in a way that protects the RNA construct. Future experiments will clarify how these two RNA regions act together to protect against exonucleolytic degradation and whether this principle can be exploited in the riboswitch design.



Acknowledgement

We thank Jasmin Walter and Niklas von Malottki for their work on the project during their bachelor theses.
We also thank Tim Kolberg for his work at the pilot project and for valuable discussion.
This work is supported by the DFG (Mo 634/24-1 to MM) and the FWF (I 6440-N to MTW)

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