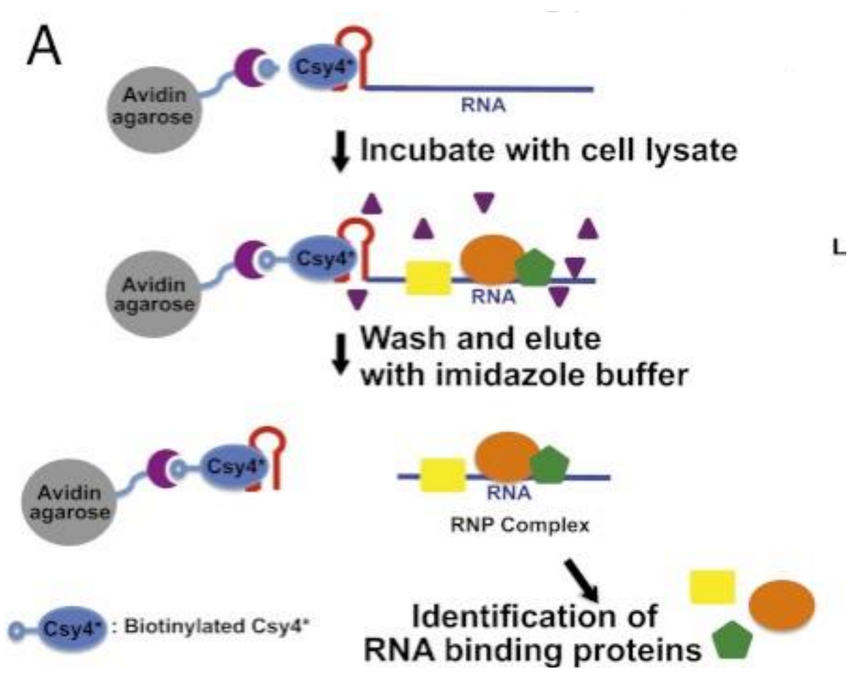
6S RNA

Sabine Schneider, Ann-Kathrin Weier
Institute for Synthetic Microbiology, Heinrich Heine Universität Düsseldorf

Abstract
Since year 1997 (Watanabe et al., 1997) it is well known that in two freshwater species of cyanobacteria 6S RNAs exist. This regulatory RNA is similar to the once found in Y-proteobacteria for example in E. coli and B. subtilis. In E. coli the 6S RNA has a converted secondary structure with consists of a single stranded central bubble within a highly double stranded molecule. This structure is essential because it mimics an open DNA promoter which then binds the E. coli RNA-polymerase. B. subtilis also contains 6S RNAs in difference to E. coli it exists two versions of 6S RNAs in B. subtilis.

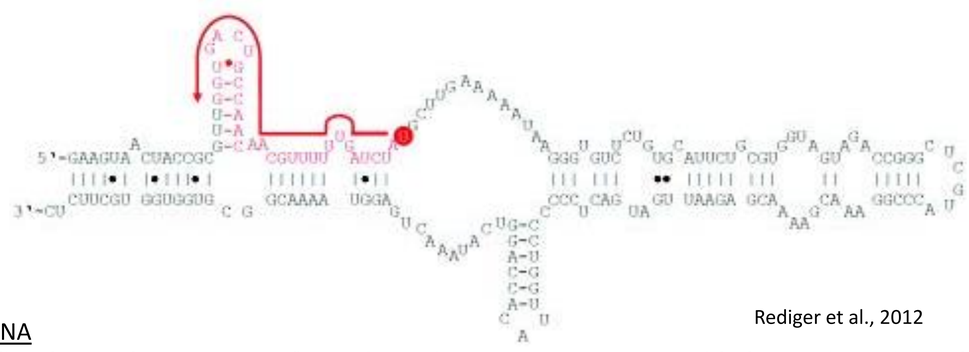
Back to the cyanobactera: The 6S RNA of Synechocystis sp. PCC 6803 has a predicted highly stable secondary structure (Barrick et al., 2005) with a possible starting point of pRNA transcription. The deletion mutant has a specific phenotype. So far it is not understood how the noncoding RNAs effect the dynamics of transcriptional networks. Therefor we are interest on protein binding partners which bind to the cyanobacteria 6S RNA of Synechocystis sp. PCC 6803. To aim this goal, we use a technology for isolating RNA protein binding partners with a CRISPR endoribonuclease called Csy4 H29A/S50C (Doudna et al., 2013). This Csy4 nuclease binds specific on a 16-nt hairpin RNA sequence at their 5` end. Afterwards this complex can be linked to the regulatory RNA. The immobilized complex gets incubated with cell proteins or other molecules. By adding imidazole the Csy4 is activated and cleavages behind the hairpin RNA so that the 6S RNA and the bound proteins are eluted.



Lee et al., 2013

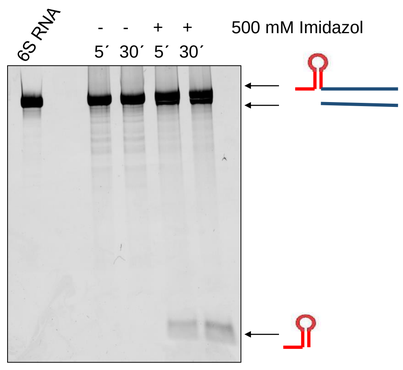
Schema of the Csy4 based RNA-protein pull down strategie

Schema of the Csy4 based RNA-protein pull down strategie
In the first step the biotinylated Csy4 protein was incubated with Avidin agarose and hairpin-tagged RNA. After to make a ternary complex, the complex was incubated with cell protein. Following washing the RNA-protein complex four/five times and eluted with hight molar imidazol buffer. Identification of RNA binding proteins with LC-MS or MALDI-TOF and visulized possibly binding partners on SDS-gel.

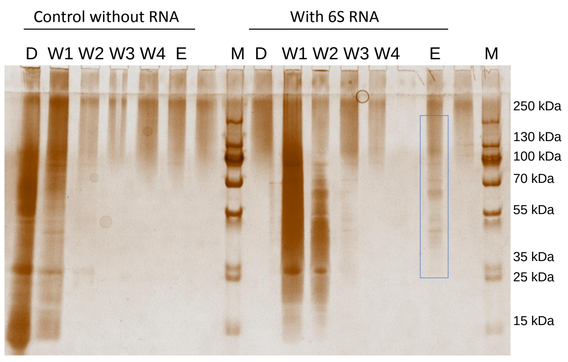


Rediger et al., 2012

6S RNA
Predicted secondary structure of Synechocystis sp. PCC 6803 6S RNA, with the starting point and direction of pRNA transcription in red



Activity test of Csy4
Cleavage essay, to check the Csy4 activity. The haippin-tagged RNA was incubated with and without 500 mM imidazol buffer. Cleavage products were seperated by denaturating 10% PAA gel. The regulatory RNA is in blue and the hairpin RNA is painted in red. Cleavage products to appear by additing hight molar imidazol buffer.



First SDS gel of the RNA-protein in vitro pull down and controll flow
6S RNA was immobilized after binding to the biotinylated Csy4 Avidin agarose beads complex. Solubly protein extract from ΔssAA cells were incubated and bound proteins eluted after several washing steps. Specifically bound protein candidates (blue barr) were analysed on SDS gel and visulised by silver staining. Further characterization by LC-MS analysed shown a shedule of possibly candidates.

Name	Stamm
Phycocyanin subunit B	Synechocystis sp. PCC 6803
Phycocyanin a subunit	Synechocystis sp. PCC 6803
Chaperonin 2	Synechocystis sp. PCC 6803
Elongation factor Tu	Synechocystis sp. PCC 6803
Allophycocyanin a chain	Synechocystis sp. PCC 6803
Allophycocyanin b chain	Synechocystis sp. PCC 6803
Phycobilisome LCM core-membrane linker polypeptide	Synechocystis sp. PCC 6803
Glyceraldehyde-3-phosphate dehydrogenase	Synechocystis sp. PCC 6803
Phycocyanin associated linker protein	Synechocystis sp. PCC 6803
Phycobilisome rod-core linker polypeptide;CpcG	Synechocystis sp. PCC 6803
Fructose-1,6-bisphosphate aldolase	Synechocystis sp. PCC 6803
Phosphoglycerate kinase	Synechocystis sp. PCC 6803
Ribulose bisphosphate carboxylase	Synechocystis sp. PCC 6803
Hypothetical protein sll1582	Synechocystis sp. PCC 6803
REVERSED transposase	Synechocystis sp. PCC 6803
REVERSED transposase	Synechocystis sp. PCC 6803
REVERSED transposase	Synechocystis sp. PCC 6803

Schedule of possibly binding partners of the regulatory 6S RNA after the first RNA-protein pull down. The eluate was analysed by LC-MS (by Tino Polen, FZ Jülich). Through the short table shown that the essay is very specific.

Dr. Tino Polen
Forschungszentrum Jülich
Institut für Bio- und Geowissenschaften
IBG-1: Biotechnologie

Outlook / Future works

- Further RNA-protein pull down with Cyano cell protein under different growth conditions (step down with nitrogen starvation and recovery) and reproductions are required
- Purification of possibly RNA binding partners and to execute EMSAs and binding structur identification
- The physiological relation of the identified proteins to bind 6S RNA is not obvious and needs further studies
- Other Csy4-based regulatory RNA affinity purification

Watanabe, T., Sugiura, M. & Sugita, M. (1997). A novel small stable RNA, 6Sa RNA, from the cyanobacterium Synechococcus sp. strain PCC6301. FEBS Lett 416, 302–306.

Barrick, J. E., Sudarsan, N., Weinberg, Z., Ruzzo, W. L. & Breaker, R. R. (2005). 6S RNA is a widespread regulator of eubacterial RNA polymerase that resembles an open promoter. RNA 11, 774–784.

Lee, H.Y., Haurwitz,R. E., Apffel,A., Zhou, K., Smart, B., Wengler, C.D., Laderman, S., Bruhn, L. and Doudna, J.A. (2013). RNA-Protein analysis using a conditional CRISPR nuclease. PNAS 110(14):5416-5421.

Rediger, A., Geissen, R., Steuten, B., Heilmann, B., Wagner, R. and Axmann, I.M. (2012)). 6S RNA – an old issue become blue-green. Microbiology 158(10):2480-91.