

Master thesis in Genetics (AG HESS)

Topic: Post-transcriptional control of specific routes in primary C metabolism of cyanobacteria

Nitrogen (N) assimilation needs to be tightly controlled within organisms to allow acclimation to external fluctuations. In the model cyanobacterium *Synechocystis* sp. PCC 6803 acclimation to N-limiting conditions involves both, the activation of multiple uptake systems for combined N-sources and of enzymes involved in specific routes of the N-assimilation. The N- and energetic status is sensed by the PII-protein which regulates the activity of the transcriptional regulator NtcA. Subsequently, NtcA controls the coordinated up-regulation of genes that counteract N-limiting conditions. In addition to transcriptional regulators, bacteria possess numerous and diverse means of gene regulation using non-coding, small RNAs (sRNAs). These sRNAs can activate or repress gene expression at posttranscriptional level, contributing to the specific and customized synthesis of the respective proteins, e.g. also under N-limiting conditions. For instance, the expression of the nitrogen stress induced RNA 4 (NsiR4) is activated by NtcA upon nitrogen limitation. NsiR4 interferes with the expression of the *gifA* gene which encodes an inhibitory protein for the glutamine synthetase. Hence, NsiR4 is involved in controlling ammonium assimilation in *Synechocystis* and probably many other cyanobacteria.

Additionally, *in silico* analyses suggested several other NsiR4 interaction partners such as mRNAs encoding enzymes of the CBB cycle, glycogen synthesis and the photosynthetic electron transport chain. All these enzymes appear to represent central hubs determining specific routes of C metabolism (**Figure 1**). Consequently, NsiR4 might not only be involved in controlling N assimilation by fine-tuning the activity of glutamine synthetase indirectly by targeting IF7/GifA. It could also influence important routes of carbon (C) metabolism by directly targeting corresponding key enzymes.

The potential Master candidate will perform detailed expression analyses of the putative NsiR4 target genes using *Synechocystis* mutants with altered NsiR4 expression levels. Moreover, the direct interaction between NsiR4 and the corresponding mRNAs will be investigated using a heterologous reporter system. This will allow the conclusion whether or not, NsiR4 directly regulates translation of several important enzymes in cyanobacterial C metabolism.

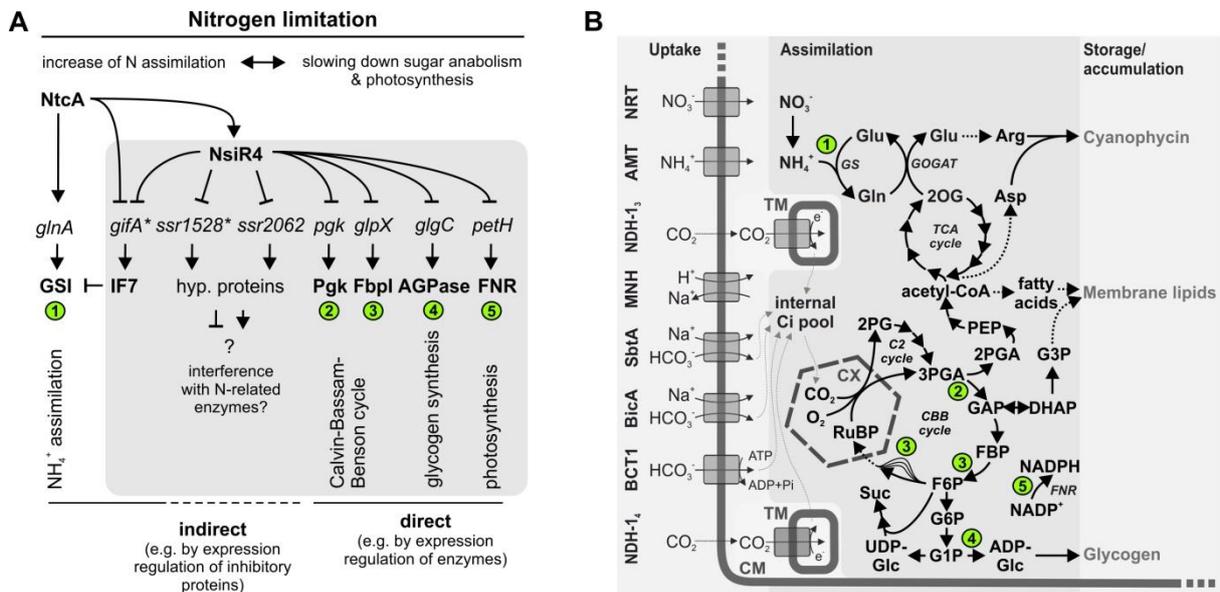


Figure 1: Functional context of predicted as well as verified NsiR4 targets. **A:** Overview of the NsiR4 regulon based on both predictions and experimental data. Experimentally verified NsiR4 targets are labeled with asterisks. The numbers highlighted in green correspond to biochemical reactions NsiR4 (putatively) interferes with (see also D). **B:** Schematic illustration of C- and N-assimilation pathways in *Synechocystis* sp. PCC 6803. Ci - inorganic carbon, CM - cytoplasmic membrane, TM - thylakoid membrane, CX - carboxysomes, 2OG - 2-oxoglutarate, Glu - glutamate, Gln - glutamine, Arg - arginine, Asp - aspartate, PEP - phosphoenol pyruvate, 2PGA - 2-phosphoglycerate, 3PGA - 3-phosphoglycerate, G3P - glycerol-3-phosphate, GAP - glyceraldehyde 3-phosphate, DHAP - dihydroxyacetone phosphate, F6P - fructose 1,6-bisphosphate, F6P - fructose 6-phosphate, 2PG - 2-phosphoglycerate, RuBP - ribulose-1,5-bisphosphate, G6P - glucose 6-phosphate, G1P - glucose 1-phosphate, UDP/ADP-Glc - UDP/ADP-glucose, Suc - sucrose, TCA cycle - tricarboxylic acid cycle, CBB cycle - Calvin-Benson-Bassam cycle, GS - glutamine synthetase, GOGAT - glutamine 2-oxoglutarate aminotransferase, FNR - ferredoxin:NADP oxidoreductase.

Related references:

Klähn S, Schaal C, Georg J, Baumgartner D, Knippen G, Hagemann M, Muro-Pastor AM, and Hess WR. (2015). The sRNA NsiR4 is involved in nitrogen assimilation control in cyanobacteria by targeting glutamine synthetase inactivating factor IF7. Proceedings of the National Academy of Sciences of the United States of America, 112 (45): E6243-E6252.

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