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Nitrogen interaction network in the cyanobacterium synechococcus WH5701, a model organism with two PipX and two PII -like proteins

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Cyanobacterial nitrogen signaling involves a complex network of protein interactions. The key regulators NtcA, a transcription factor member of the Crp/CAP superfamily, and the signal transduction protein PII, perceive nitrogen status by sensing intracellular 2-oxoglutarate levels. PII proteins are remarkable for their ability to interact with very diverse protein targets in different systems. In oxygenic photosynthetic organisms, PII forms complexes with NAGK, an enzyme involved in arginine biosynthesis. In cyanobacteria, P_{II} also interacts with PipX, a protein with a tudor-like domain that mediates contacts with P_{II} and with the transcriptional regulator NtcA, to which it binds to increase its activity. Recently, we showed that PipX is toxic in PII deficient cultures of *S. elongatus*. Interestingly, 3 sequenced cyanobacteria (*Synechococcus* sp. WH5701, *Acaryochloris marina* MBIC11017, *Gloeobacter violaceus* PCC 7421) with two *pipX*-like genes also contain a second copy of *glnB* (encoding PII). Single copies of *ntcA* and *argB* (encoding NAGK) are found in all cyanobacteria. In this work we used a combination of *in silico*, yeast two-hybrid and *in vitro* approaches to investigate the nitrogen regulation network of *Synechococcus* WH5701, a marine cyanobacterium with two PII (GlnB_A and GlnB_B) and two PipX (PipX_I and PipX_II) proteins. Our results show that GlnB_A and PipX_II are functionally equivalent to PII and PipX from the model cyanobacterium *S. elongatus*. PipX_II, and to a lesser extent PipX_I, specifically interacted with GlnB_A and NtcA, in agreement with the idea that at least PipX_II would mediate partner swapping between GlnB_A and NtcA according to the intracellular 2-OG levels.