

Proteoma (PRO)

Mapping of the interactome of PII family proteins from the free living diazotroph *Azospirillum amazonense*

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The PII superfamily of signal transduction proteins is known to contain the most widely distributed signaling proteins in nature, being ubiquitous among bacteria. They play a major role in coordinating the regulation of central metabolic processes, especially those related to nitrogen metabolism. PII proteins act by direct interaction with a variety of cellular targets, such as transcriptional factors, enzymes and transporters, resulting in modulation of receptor activities. PII signaling has the potential to respond to three central metabolic signals: the energy signal ATP, the carbon signal 2-ketoglutarate (2-KG) and the nitrogen signal glutamine. The first two are sensed directly by the binding of the effectors molecules. The nitrogen levels are reflected by reversible uridylation of PII by the glutamine-sensor enzyme GlnD, under nitrogen deficiency conditions. ^[1,2]

In diazotrophic microorganisms, PII proteins role is extended to the control of the nitrogen fixation process. The genus *Azospirillum* is composed of free-living diazotrophs capable of beneficial association with a variety of Graminae plants, such as sugarcane and rice. In order to appraise the regulatory systems of nitrogen fixation and metabolism in the model organism *Azospirillum amazonense*, we propose an investigation of the PII proteins function in these networks. The cellular targets of each of the two PII paralogues found in *A. amazonense* – named GlnB and GlnK – are under investigation by pull down assays. Two protein extracts of *A. amazonense* are being tested, referring to growth conditions of nitrogen limitation and sufficiency. The interacting proteins are identified by LC-MS/MS, and interactions are to be further confirmed by alternative methods, such as yeast two-hybrid system. Up to this moment, several potential interacting partners of non-uridylated GlnB and GlnK were identified. Some of these proteins are predicted to be associated with amino-acid biosynthesis, fructose metabolism, transcription-repair coupling and conjugation. Others are though to fulfill regulatory roles.

Furthermore, we wish to compare the specific targets of the uridylated and non-uridylated forms of PII. Hence, the purified recombinant PII proteins will be uridylated *in vitro* by the GlnD recombinant enzyme of *Azospirillum brasilense*, prior to pull-down experiments. This post-translational modification will be verified by mass-spectrometry, in a MALDI-TOF instrument in linear positive mode. Finally, it is also of our interest to investigate the influence of ATP and 2-KG levels in such interactions.

Support: CAPES, CNPq, Fapergs

[1] Forchhammer, K. *Trends Microbiol.* **2008**, *16*(2), 65-72.

[2] Jiang P, Ninfa A. J. *Biochemistry.* **2009**, *48*,11522-31.