AMMONIUM-INDUCED NITROGENASE INACTIVATION IS REGULATED BY P_{II} PROTEINS AND AMTB- DEPENDED MEMBRANE SEQUESTRATION OF A REGULATORY ENZYME IN *AZOSPIRILLUM BRASILENSE*

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The free-living nitrogen-fixing bacteria *Azospirillum brasilense* has attracted considerable attention due to its capacity of colonize the roots and enhance the growth and yield of several economically important crops worldwide. Nitrogenase activity of *A. brasilense* is reversibly inactivated by NH_4^+. This inactivation involves ADP-ribosylation of the Fe-protein (dinitrogenase reductase) catalysed by the dinitrogenase reductase ADP-ribosyltransferase (DraT) and is reversed, upon NH_4^+ exhaustion, by dinitrogenase reductase activating glycohydrolase (DraG). The activities of both DraT and DraG are regulated accordingly to the external levels of ammonium. Previously genetic data has suggested that proteins from the signal transduction family P_{II} and ammonia channel AmtB are involved in the regulation of DraT and/or DraG activities though the signalling pathway has not unravelled yet. Here we show that the *A. brasilense* P_{II} proteins (namely GlnB and GlnZ) interact with the nitrogenase regulatory enzymes DraT and DraG, and these interactions are regulated not only by the cellular nitrogen levels but also by energy and carbon signals. We also observed that DraG is reversibly sequestered to the cell membrane accordingly to the ammonium availability by the formation of an AmtB-GlnZ-DraG ternary complex. These observations led us to propose a model for the signalling pathway leading to the ammonium elicited nitrogenase inactivation in *A. brasilense*.

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