## Interaction of the general transcription factor TnrA with the PII-like protein GlnK and glutamine synthetase in Bacillus subtilis

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## Abstract

TnrA is a master transcription factor regulating nitrogen metabolism in Bacillus subtilis under conditions of nitrogen limitation. When the preferred nitrogen source is in excess, feedbackinhibited glutamine synthetase (GS) has been shown to bind TnrA and disable its activity. In cells grown with an energetically unfavorable nitrogen source such as nitrate, TnrA is fully membrane-bound via a complex of AmtB and GlnK, which are the transmembrane ammonium transporter and its cognate regulator, respectively, originally termed NrgA and NrgB. The complete removal of nitrate from the medium leads to rapid degradation of TnrA in wild-type cells. In contrast, in AmtB-deficient or GInK-deficient strains, TnrA is neither membrane-bound nor degraded in response to nitrate depletion. Here, we show that TnrA forms either a stable soluble complex with GInK in the absence of AmtB, or constitutively binds to GS in the absence of GInK. In vitro, the TnrA C-terminus is responsible for interactions with either GS or GInK, and this region appears also to mediate proteolysis, suggesting that binding of GlnK or GS protects TnrA from degradation. Surface plasmon resonance detection assays have demonstrated that GS binds to TnrA not only in its feedback-inhibited form, but also in its non-feedback-inhibited form, although less efficiently. TnrA binding to GlnK or GS responds differentially to adenylate nucleotide levels, with ATP weakening interactions with both partners. Structured digital abstract to by () to by () to by () to by () with by () to by () with by () with by () with by () to by () to by () The present paper reveals a novel mechanism for regulating the stability of the general nitrogen-stress transcription factor TnrA in Bacillus subtilis. TnrA remains resistant to intracellular proteolysis as long as it is complexed to either GlnK or glutamine synthetase (GS). Interaction with both proteins occurs via the C-terminus of TnrA, which is also recognized by the proteolytic activity © 2011 The Authors Journal compilation.

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## **Keywords**

Bacillus subtilis, GlnK, glutamine synthetase, nitrogen regulation, PII protein, transcription factor TnrA