Interaction of the general transcription factor TnRA with the PII-like protein GlNK and glutamine synthetase in Bacillus subtilis

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Bacillus subtilis; GlNK; glutamine synthetase; nitrogen regulation; PII protein; transcription factor TnRA

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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, feedback-inhibited 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 GlNK-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 GlNK in the absence of AmtB, or constitutively binds to GS in the absence of GlNK. In vitro, the TnRA C-terminus is responsible for interactions with either GS or GlNK, 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

- **tnRA** binds to glnk by **surface plasmon resonance** (View interaction)
- GS binds to **tnRA** by **pull down** (View interaction)
- **tnRA** binds to glnk by **pull down** (View interaction)
- **tnRA** binds to GS by **pull down** (View interaction)
- GS physically interacts with **tnRA** by **anti bait commounprecipitation** (View interaction)
- glnk binds to **tnRA** by **pull down** (View interaction)
- glnk physically interacts with **tnRA** by **anti bait commounprecipitation** (View interaction)
- **tnRA** physically interacts with GS by **anti bait commounprecipitation** (View interaction)
- **tnRA** physically interacts with glnk by **anti bait commounprecipitation** (View interaction)
- **tnRA** binds to **tnRA** by **cross-linking study** (View interaction)
- **tnRA** binds to GS by **surface plasmon resonance** (View interaction)

Abbreviations
FC, flow cell; Glnk-ST, Strep-tag II-tagged variant of GlNK; GS, glutamine synthetase; GS-ST, Strep-tag II-tagged variant of glutamine synthetase; ITC, isothermal titration calorimetry; NAGK, N-acetylglutamate kinase; SPR, surface plasmon resonance.