

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

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## Keywords

*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 coimmunoprecipitation](#) ([View interaction](#))
- [glnK binds to tnrA](#) by [pull down](#) ([View interaction](#))
- [glnK physically interacts with tnrA](#) by [anti bait coimmunoprecipitation](#) ([View interaction](#))
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- [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*-acetyl-L-glutamate kinase; SPR, surface plasmon resonance.