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Transcription factor TnrA inhibits the biosynthetic activity of glutamine synthetase in *Bacillus subtilis*

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ABSTRACT

The *Bacillus subtilis* glutamine synthetase (GS) plays a dual role in cell metabolism by functioning as catalyst and regulator. GS catalyses the ATP-dependent synthesis of glutamine from glutamate and ammonium. Under nitrogen-rich conditions, GS becomes feedback-inhibited by high intracellular glutamine levels and then binds transcription factors GlnR and TnrA, which control the genes of nitrogen assimilation. While GS-bound TnrA is no longer able to interact with DNA, GlnR–DNA binding is shown to be stimulated by GS complex formation. In this paper we show a new physiological feature of the interaction between glutamine synthetase and TnrA. The transcription factor TnrA inhibits the biosynthetic activity of glutamine synthetase *in vivo* and *in vitro*, while the GlnR protein does not affect the activity of the enzyme.

Structured summary of protein interactions:
GS physically interacts with **TnrA** by anti bait coimmunoprecipitation (View interaction)
TnrA binds to **GS** by pull down (View interaction)
TnrA binds to **GS** by surface plasmon resonance (View interaction)
GlnK physically interacts with **TnrA** by anti bait coimmunoprecipitation (View interaction)
GlnK binds to **GS** by pull down (View interaction)

1. Introduction

Glutamine synthetase (GS) is a metalloenzyme catalyzing the ATP-dependent synthesis of glutamine from glutamate and ammonium [1,2]. This is a universal reaction for many bacteria and is the predominant mechanism by which inorganic nitrogen is incorporated into cellular metabolites. Since *Bacillus subtilis* does not contain an anabolic glutamate dehydrogenase [3], this is also the only pathway for ammonium assimilation in *B. subtilis*. Glutamine is a key compound in nitrogen metabolism and the synthesis as well as activity of GS is strongly regulated by a variety of mechanisms in response to nitrogen availability. The activity of GS is high in cells growing under nitrogen-limiting conditions and is low when cells are growing rapidly with nitrogen excess [4].

GS from *B. subtilis* is not regulated by any known post-translational protein modification [5], but is directly feedback-inhibited by glutamine and several end-products of glutamine metabolism [6]. In addition to glutamine, AMP is the second most effective feedback inhibitor of GS. Glutamine binds to the glutamate substrate site whereas AMP binds at the ATP site [6]. The glutamine biosynthetic activity of *B. subtilis* GS can be assayed *in vitro* and requires Mg or Mn^{2+} [7]. Mg -dependent biosynthetic reaction is the main physiologically relevant enzymatic activity [1,2] and can be completely inhibited by glutamine or AMP. The Mn^{2+}-dependent biosynthetic activity is only partially inhibited by alanine, serine or glycine.

B. subtilis GS is a trigger enzyme and has a dual function: as a biosynthetic enzyme for ammonium assimilation and glutamine synthesis and as a regulatory enzyme controlling the activity of transcription factors by direct protein–protein interactions [8,9]. Two transcription factors, termed GlnR and TnrA, control the expression of nitrogen-regulated genes in *B. subtilis* [10,11]. Both of these proteins are members of the MerR family of transcription regulators, contain a helix-turn-helix DNA-binding domain at the N-terminus and bind to the same DNA-consensus sequence [11,12]. Although the sequences of the amino-terminal DNA-binding domains of TnrA and GlnR are highly similar, these proteins have little sequence similarity in their C-terminal signal transduction

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