DOI 10.1099/mic.0.2008/019802-0

Inactivation of the general transcription factor TnrA in *Bacillus subtilis* by proteolysis

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Received 9 April 2008
Accepted 6 May 2008

Under conditions of nitrogen limitation, the general transcription factor TnrA in *Bacillus subtilis* activates the expression of genes involved in assimilation of various nitrogen sources. Previously, TnrA activity has been shown to be controlled by protein–protein interaction with glutamine synthetase, the key enzyme of ammonia assimilation. Furthermore, depending on ATP and 2-oxoglutarate levels, TnrA can bind to the GlnK–AmtB complex. Here, we report that upon transfer of nitrate-grown cells to combined nitrogen-depleted medium, TnrA is rapidly eliminated from the cells by proteolysis. As long as TnrA is membrane-bound through GlnK–AmtB interaction it seems to be protected from degradation. Upon removal of nitrogen sources, the localization of TnrA becomes cytosolic and degradation occurs. The proteolytic activity against TnrA was detected in the cytosolic fraction but not in the membrane, and its presence does not depend on the nitrogen regime of cell growth. The proteolytic degradation of TnrA as a response to complete nitrogen starvation might represent a novel mechanism of TnrA control in *B. subtilis*.

INTRODUCTION

Nitrogen is a macronutrient for all known types of living organisms, since it is included in most biomolecules. In natural environments, the nitrogen sources may differ in both composition and concentration. To optimize their utilization, bacteria have developed regulatory systems that control gene expression in response to changes in the nitrogen availability (reviewed by Forchhammer, 2007).

TnrA is the major transcription factor in *Bacillus subtilis* that controls gene expression in response to nitrogen availability. Under nitrogen-limited growth, TnrA binds to a dyad symmetry element with the consensus sequence 5′-TGTNAN7TNACA-3′ (Wray *et al.*, 1997, 2000), and serves as either an activator or a repressor of genes. TnrA activates its own gene (Fisher, 1999), the *nasABCDEF* genes (nitrate and nitrite utilization; Nakano *et al.*, 1995, 1998), the *nrgAB* (*amtBglnK*) operon (ammonium transport; Wray *et al.*, 1998), the *ureABC* operon (urea utilization; Wray *et al.*, 1997) and the genes for purine utilization, and interacts with some other target promoters. Under nitrogen-limited growth, TnrA is a negative regulator of *glnA* and *gltAB*, which encode the ammonium assimilatory enzymes glutamine synthetase (GS) and glutamate synthase, respectively (Wray *et al.*, 1996; Fisher & Debarbouille, 2002; Belitsky *et al.*, 2000). Interestingly, the induction of the *gltAB* operon depends on the pleiotropic regulator of carbon metabolism CcpA, and requires sugars that can be catabolized via glycolysis (Faires *et al.*, 1999; Blencke *et al.*, 2003; Wacker *et al.*, 2003).

Several lines of evidence indicate that GS acts as a sensor of nitrogen availability in *B. subtilis* (Fisher, 1999). TnrA-activated genes are expressed constitutively in *glnA* mutants, implying that GS produces or transmits an inhibitory regulatory signal to TnrA during growth with excess nitrogen (Fisher *et al.*, 2002). Indeed, the feedback-inhibited GS forms a complex with TnrA, preventing its binding to DNA (Wray *et al.*, 2001). The most effective feedback inhibitors of GS biosynthetic activity are glutamine and AMP, while partial inhibition has been observed with alanine, glycine, serine and tryptophan (Deuel & Prusiner, 1974). Mutations in TnrA that result in constitutive expression of the TnrA-activated *amtB* promoter all lie within the carboxy-terminal region of TnrA and impair the interaction between GS and TnrA (Wray *et al.*, 2001; Wray & Fisher, 2006). Thus, the feedback inhibitors of GS are the metabolic signals that cause inhibition of TnrA through its GS interaction.

Other regulators of TnrA activity have been found recently. When *B. subtilis* cells were grown with the poor nitrogen source nitrate, TnrA was found, in cell-free extracts, to be almost completely associated with the cell membrane via the ammonium-uptake proteins AmtB and GlnK, originally termed NrgA and NrgB, respectively (Heinrich *et al.*,

Abbreviations: GS, glutamine synthetase; NAG-kinase, *N*-acetyl-L-glutamate kinase.

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