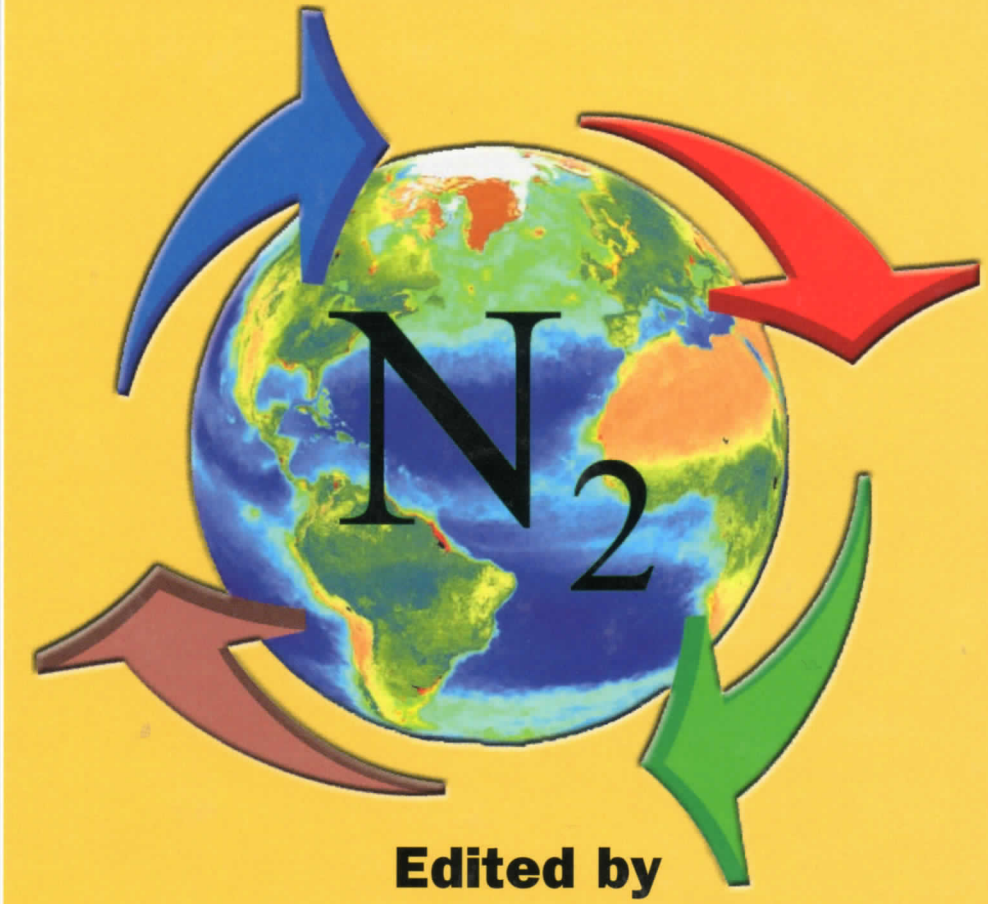
NITROGEN FIXATION

Global Perspectives



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EFFECT OF TRUNCATIONS AND CONSTITUTIVE NtrB MUTATIONS IN MOLECULAR INTERACTIONS WITHIN THE *KLEBSIELLA PNEUMONIAE* NITROGEN-SIGNAL TRANSDUCTION PATHWAY

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1. Introduction

In *Klebsiella pneumoniae*, signal transduction in response to nitrogen availability is mediated by the two-component regulators NtrB and NtrC. NtrB, a bifunctional histidine-kinase, modulates the activity of the response regulator NtrC by phosphorylation. The ability of NtrB of switching between opposing kinase and phosphatase activities is regulated by PII, a trimeric protein interacting with NtrB in conditions of nitrogen sufficiency. In a previous work, we showed the usefulness of the yeast two-hybrid system to probe NtrB and NtrC homodimerization and also specific interactions between NtrC receiver and NtrB transmitter domains (Martínez-Argudo *et al.* 2001). Here we use the same *in vivo* strategy to further analyze interactions of NtrB within the nitrogen signal pathway, probing NtrB determinants for interactions with itself, NtrC and PII, and testing the effect of NtrB constitutive point mutations on these interactions.

2. Results and Discussion

Protein fusions of GAL4-AD and GAL4-BD to NtrB and derived polypeptides (including truncations and a constitutive mutation at A129T) were analyzed for their ability to interact with themselves and with equivalent fusions of signal transduction proteins PII and NtrC, in appropriate pairs, using the yeast two-hybrid system. To determine the ability of two given polypeptides to interact, we determined expression of both *GAL1:lacZ* and *GAL1:HIS3* reporters in strains of *Saccharomyces cerevisiae* Y190.

Protein-protein interactions were only detected between components of the nitrogen signal pathway. Lack of signals between heterologous two-component regulators provides evidence for specific recognition between the transmitter module of NtrB and both PII and NtrC. Contacts of NtrB with NtrC and PII are mapped to the H phosphotransfer domain and to the G kinase domain, respectively. In the latter case, the integrity of the transmitter module appears important for two-hybrid signals. Taken together, our results agree with previous data on homologous two-component systems (Park *et al.* 1998) and with recent work on PII (Piozak *et al.* 2000).

In spite of the multiple evidences for dimerization of phosphotransfer domains (Tomomori *et al.* 1999; Jiang *et al.* 2000), transmitter modules from NtrB and EnvZ do not interact when paired with themselves, a result that may reflect that contacts between H domains are not very strong. This would be compatible with a model in which the helix bundle forms and dissociates during the phosphorylation circle.

Mutation A129T affects some of the interactions tested amongst NtrB derivatives, thus supporting its effect on NtrB conformation and the sensitivity of the two-hybrid system used here.

3. References

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