PII T-Loop Mutations Affecting Signal Transduction to NtrB Also Abolish Yeast Two-Hybrid Interactions

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PII proteins play a key role in both transcriptional regulation and metabolic regulation in bacteria, being among the most ubiquitious signal transduction proteins (reviewed in reference 1). Two paralogous genes, glnB and the more recently discovered gene glnK, encode PII proteins (referred to as GlnB and GlnK, respectively) in enterobacteria. The physical properties of the Escherichia coli GlnB and GlnK proteins are very similar, and under some circumstances, GlnK can substitute for GlnB. The structures of the E. coli PII proteins are known to be very high resolution (16, 17).

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yeast culture and transformation procedures used were as previously described (2). β-Galactosidase activity was assayed as previously described (14). Growth on histidine-deficient medium was analyzed on solid YNB medium lacking His, Leu, and Trp in the presence of different concentrations of 3-aminotriazole, and growth rates were categorized into four classes (+, +, ±, and −) as previously described (10). The fusion proteins carried by a given strain are always named in the order GAL4AD-X/GAL4BD-Y, which is abbreviated X/Y, where X or Y is any polypeptide fused to GAL4AD and GAL4BD, respectively. Following previously established conventions, the transmitter module of NtrB is designated NtrB\(\text{HNG}\). For simplicity, GlnB derivatives are designated PII conventions, where X or Y is any polypeptide fused to GAL4AD and GAL4BD, respectively. Following previously established conventions.

When mutant and wild-type versions of PII were tested for the ability to interact with NtrB or NtrB\(\text{HNG}\), both PII\(\text{ATP}^{-}\) and PII\(\text{A49P}^{-}\) failed to give signals with the two reporter genes while significant signals were always obtained with PII (Fig. 1). GlnB wild-type fusion proteins from both \(K.\ pneumoniae\) and \(E.\ coli\) were assayed with identical results (data not shown). Therefore, these results closely parallel the failure of the corresponding mutant PII proteins to regulate NtrB both in vivo and in vitro (9). To exclude a negative impact of the T-loop mutations on the stability of fusion proteins in yeast, their expression was analyzed by Western blot assays. As shown in Fig. 2, expression of mutant PII derivatives in yeast is as good as that of their wild-type counterpart, thus indicating that the lack of interaction in the two-hybrid system observed with PII\(\text{A49P}^{-}\) and PII\(\text{ATP}^{-}\) is not due to reduced stability of the corresponding fusion proteins in the yeast system and suggesting that the inability of the corresponding mutant proteins to elicit NtrB phosphatase activity does, indeed, reflect binding impairment. The fact that PII\(\text{A49P}^{-}\) specifically and completely abolished interaction with NtrB and NtrB\(\text{HNG}\) in the two-hybrid system suggests either that there are no additional determinants outside the T loop or that they are not important enough to overcome the deleterious effect of the A49P change on NtrB recognition.

It is worth noting that, with the exception of its ability to regulate NtrB, the A49P point mutation does not affect any known property or function of PII. In fact, and in spite of the close proximity of Ala49 to Tyr51, the active site for the uridylyl transfer reaction, PII\(\text{A49P}^{-}\) is uridylylated as efficiently as PII (9). The specific impact of the A49P point mutation on interactions with NtrB is also observed in the yeast two-hybrid system, since UTase/PII and UTase/PII\(\text{A49P}^{-}\), but not UTase/PII\(\text{ATP}^{-}\), gave signals with both the \(GAL1::\text{lacZ}\) and \(GAL1::\text{HIS3}\) reporters (data not shown).

In addition to establishing, for particular PII mutants, a clear

![FIG. 1. Effects of PII T-loop mutations on expression of \(GAL1::\text{lacZ}\) and \(GAL1::\text{HIS3}\) in strain Y190 carrying different pairs of fusion proteins. Dashes indicate the absence of proteins fused to GAL4 domains. The numbered lines between panels A and B encompass blocks of data according to the type of comparison: 1, PII derivatives paired with GAL4BD-NtrB; 2, PII derivatives paired with GAL4AD-NtrB; 3, PII derivatives paired with GAL4BD::NtrB\(\text{HNG}\); 4, PII derivatives paired with GAL4AD::NtrB\(\text{HNG}\). (A) Each bar represents the mean β-galactosidase activity of at least four independent transformants, each measured in triplicate. (B) Levels of growth on histidine-deficient medium.](image-url)
correlation between regulation of NtrB and the ability to interact in the two-hybrid system, our results indicate that the yeast two-hybrid system used here may be particularly fruitful when used to study interactions mediated by T-loop-containing proteins such as PII, further validating the use of yeast two-hybrid approaches to address two different biological problems: the study of specific interactions with known PII receptors by direct and reverse strategies and the identification of additional receptors for PII proteins. Given the growing interest in the function of PII proteins from a great variety of organisms, for the majority of which there are no genetic tools available, it can be anticipated that the use of PII as bait to screen genomic libraries would greatly facilitate the task of identifying PII receptors.

FIG. 2. Western blotting of GAL4BD fused to PII and mutant derivatives. In each case, protein extracts were derived from 2- to 3-ml cultures (turbidity at 600 nm, 2) of Y190 also carrying GAL4AD-NtrB. Protein extracts were treated, separated, transferred to the membrane, and probed with a monoclonal antibody to GAL4BD (sc-510) from Santa Cruz Biotechnology as previously described (11). An arrowhead points to the protein indicated above each lane. The values to the left are molecular masses (in kilodaltons) of protein size standards.

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REFERENCES


