A glutamine-amidotransferase-like protein modulates FixT anti-kinase activity in Sinorhizobium meliloti

ABSTRACT

Isolation of the asnO mutant argues for the existence of a physiological regulation associated with fixT and makes it unlikely that fixT serves a mere homeostatic function in S. meliloti. Our data suggest that asnO might control activity of the FixT protein, in a way that remains to be elucidated. A proposed role for asnO might be to couple nitrogen fixation gene expression in S. meliloti to the nitrogen needs of the cells.

INTRODUCTION

Background Sinorhizobium meliloti forms N2-fixing nodules on the roots of alfalfa (Medicago sativa) and closely related plants. Expression of nitrogen fixation genes is under both positive and negative control. This regulation depends on a regulatory cascade, on top of which the two-component regulatory system FixLJ activates expression of nitrogen fixation genes in response to microoxic conditions, such as those that prevail inside the nodule. Under microoxic conditions, the sensor histidine kinase FixL autophosphorylates and transfers its phosphate to the FixJ transcriptional regulator protein. Phosphorylated FixJ then activates transcription of two intermediate regulatory genes, nifA and fixK, that both encode transcriptional regulators. NifA mediates activation of nif genes involved in nitrogenase biosynthesis whereas FixK, a member of the Crp/Fnr family, activates expression of genes involved in the synthesis of a respiratory oxidase complex. fixK is also indirectly responsible for negative regulation of the cascade since it controls expression of a gene, fixT, that negatively affects expression of FixLJ dependent genes (see Figure 6). We have shown recently that the FixT protein negatively affects the expression of nifA and fixK by inhibiting phosphorylation of the sensor hemoprotein kinase FixL and, by consequence, phosphorylation of FixJ. Whether FixT serves a mere homeostatic function in S. meliloti (the level of FixT protein feed-back controlling activity of the FixLJ system) or whether FixT allows integration of a physiological signal by the FixLJ system was so far unknown. We addressed this question by looking for S. meliloti mutants in which the FixT protein would not be active in repression. Here we report the isolation of a S. meliloti mutant strain that phenotypically escapes the repressor activity exerted by FixT. The mutation lies in a gene named asnO encoding a protein homologous to glutamine-amidotranferases. We discuss the significance of this finding with respect to the regulation of symbiotic nitrogen fixation.

CONCLUSION

Conclusions FixT is an intriguing protein as it has not been described so far in any other bacterium besides S. meliloti. Furthermore its mode of action is original, as it has the capacity to block phosphorylation, and hence activity, of the FixL sensor histidine kinase. They are only a few examples of such anti-kinase proteins in the literature. Lastly, FixT primary sequence did not provide clues to its function. There is thus a great deal of interest in determining the biological role of fixT in S. meliloti. The present work argues in favor of a physiological function associated with fixT, by showing that mutation of the asnO gene impairs repression by the FixT protein. This finding brings support to the previous suggestion that FixT may allow integration of an additional signal by the FixLJ two-component regulatory system whose activity is primarily regulated by oxygen (Figure

6). Multiple signal integration by a single two-component regulatory system is well documented for instance in B. subtilis. Further work is required to elucidate the relationship between fixT, fixL and asnO. We propose as a working model that the absence of AsnO may result in an imbalance in the pool of a metabolite (e.g a substrate or a product of AsnO), that would affect the intrinsic repressing activity of FixT or, equally, the interaction between FixT and FixL. Identification of the reaction catalyzed by AsnO and further elucidation of the mode of action of the FixT protein should shed light to this model. Because glutamine, a likely by-product of nitrogen fixation in symbiotic rhizobia, is a predicted substrate of the AsnO protein, it is tempting to speculate that asnO and fixT may provide a link between the nitrogen status of bacteria -or of the plant cell- and nitrogen fixation activity and reducing power generation. Possibly, such a genetic device may connect the nitrogen needs of the plant to the nitrogen fixation activity of the microsymbiont.