Rhizobium meliloti Regulatory Gene fixJ Activates Transcription of R. meliloti nifA and fixK Genes in Escherichia coli

CÉCILIA HERTIG,† RUO YA LI, ANNE-MARIE LOUARN,‡ ANNE-MARIE GARNERONE, MICHEL DAVID, JACQUES BATUT, DANIEL KAHN, AND PIERRE BOISTARD*  
Laboratoire de Biologie Moléculaire des Relations Plantes-Microorganismes, Centre National de la Recherche Scientifique-Institut National de la Recherche Agronomique, Groupement Scientifique Microbiologie Toulouse, BP 27, 31326 Castanet-Tolosan Cedex, France

Received 14 June 1988/Accepted 1 December 1988

When present in Escherichia coli on the multicopy expression vector pUC19, a Rhizobium meliloti regulatory gene, fixJ, belonging to a two-component regulatory system, activated the expression of two R. meliloti symbiotic genes, nifA and fixK. Primer extension by reverse transcription showed that FixJ stimulates nifA expression in E. coli by activating pnfA.

We have recently identified a pair of regulatory genes, fixL and fixJ, which control the expression of nif and fix genes in Rhizobium meliloti either in the endosymbiotic state or in pure culture under microaerobic conditions (4). Genetic analysis showed that both fixL and fixJ are involved in the expression of nif genes because they are required for the expression of the positive activator nifA (4). Similarly, nifA-independent fix genes such as fixN need fixL and fixJ in order to be expressed (4). In this latter regulatory pathway, fixL and fixJ activate the expression of the positive regulatory gene fixK, which in turn is required for the expression of nifA-independent fix genes (J. Batut, M. L. Daveran-Mingot, M. David, J. Jacobs, and D. Kahn, manuscript submitted for publication).

FixL and FixJ proteins belong to a conserved family of two-component regulatory proteins (4). A model has been proposed to account for the properties of these systems as well as for the amino acid sequence data of the constituent proteins (11): the FixL homologs are sensor proteins which respond to an environmental factor by activating their respective FixJ homologs, which generally act as transcriptional activators. Although conserved in their N-terminal domain, which interacts with the sensor protein, the activator proteins differ in their central and C-terminal domains, which allow us to define three classes which very likely correspond to different mechanisms of transcription activation. FixJ and UhpA (15, 16), the positive regulator of the sugar phosphate transport gene uhpT, constitute one of those classes (4). Here we report experiments on the control of the expression of R. meliloti nifA and fixK in Escherichia coli. Our results support the prediction that FixJ is a transcriptional activator and furthermore suggest that FixJ could directly interact with fixK and nifA upstream regulatory sequences.

To study the expression of nifA and fixK in E. coli, we used the nifA-lacZ translational fusion plasmid pCHK57 (8) and a fixK-lacZ translational fusion plasmid, pJ5 (Batut et al., submitted), a derivative of pJJ1363 (12). Expression of fixJ in E. coli was obtained by cloning an AccI-BglII fragment of 1,524 base pairs from pDDS (5) into the polylinker of pUC19 downstream of the lac promoter (17). This fragment, between coordinates 2066 and 3590 of the fixLJ map (4), contains the entire fixJ coding sequence. The recombinant plasmid pCH2 was introduced by transformation in E. coli MC240(pCHK57) and TB1(pJ5S). β-Galactosidase activity was measured as described before, after the cells were grown in M9 medium at 28°C (4). Table 1 shows the effect of pCH2 on the expression of pCHK57 and pJ5. The nifA and fixK genes expressed under microaerobic conditions in R. meliloti (4, 8; Batut et al., submitted), we looked at their expression in E. coli either immediately after growth in aerated liquid medium or after an additional period of 1 to 3 h, during which a mixture of 98% nitrogen–2% oxygen was bubbled into the culture (4). The presence of pCH2 results in a 50- to 100-fold increase of nifA expression and a several-thousandfold increase in the expression of fixK. In both cases this stimulation was independent of the aeration status of the culture. When pJ5S and pCH2 were present in strain TG1, which carries the lac repressor gene lacP (7, 14), the expression of the fusion was dependent on the addition of the inducer isopropyl-β-d-thiogalactopyranoside (data not shown). Since in pCH2 fixJ is located downstream of the lac promoter, this was an indication that the observed expression was due to FixJ.

To see whether in E. coli the FixJ protein was activating nifA at the same promoter as in R. meliloti, we used primer extension by reverse transcriptase to detect and characterize the nifA transcript produced under the control of pCH2 in E. coli. MC240 carrying the nifA-lac fusion plasmid pCHK57.

RNA was extracted from 100-ml cultures by the method of Aiba et al. (1). Primer extension with reverse transcriptase was performed as described by Débarbouillé and Raibaud (6), by using a 20-mer oligonucleotide complementary to nucleotides 10 to 29 of the coding sequence of nifA (13). A dideoxy-sequencing reaction was performed with the same oligonucleotide as a primer and DNA from a M13 phage carrying pCHK57 insert as a template. An RNA produced in MC240(pCHK57, pCH2) but not in MC240(pCHK57) allows an extension of the primer, resulting in a fragment which comigrates with a T-terminated sequencing reaction product (Fig. 1). The complementary adenosine residue is located 53 nucleotides upstream of the ATG initiation codon. The nifA transcription start site thus determined is the same as that observed in R. meliloti either in the endosymbiotic state or in microaerobic conditions (3, 13).
TABLE 1. Expression of nifA and fixK-lacZ fusion

<table>
<thead>
<tr>
<th>Strain*</th>
<th>U of β-galactosidase/μg of protein produced in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerated culture</td>
</tr>
<tr>
<td>MC240</td>
<td>1.0</td>
</tr>
<tr>
<td>MC240(pCH2)</td>
<td>0.7</td>
</tr>
<tr>
<td>MC240(pCHK57)</td>
<td>1.7</td>
</tr>
<tr>
<td>MC240(pCHK57, pCH2)</td>
<td>216</td>
</tr>
<tr>
<td>TB1</td>
<td>0.9</td>
</tr>
<tr>
<td>TB1(pCH2)</td>
<td>0.3</td>
</tr>
<tr>
<td>TB1(pJ5)</td>
<td>0.5</td>
</tr>
<tr>
<td>TB1(pJ5, pCH2)</td>
<td>2,990</td>
</tr>
</tbody>
</table>

* MC240: ara Δ(lac-pro) naiA metB argEAm Rif supF (9); TB1: ara Δ(lac-pro) strA thi Δ80 lacZΔM15 hsdR (2).

Our data show that when cloned on a multicopy expression vector, fixJ is able to activate both nifA and fixK in E. coli. The fact that FixJ is able to activate R. meliloti nifA and fixK in the taxonomically distant species E. coli is a strong indication that FixJ directly interacts with upstream regulatory sequences of nifA and fixK. The primer extension experiment further suggests that in the case of nifA, FixJ interacts with the same upstream sequences as those recognized in R. meliloti during symbiosis. Alternatively explaining our results by a mechanism of indirect activation would require that FixJ be responsible for the production of an E. coli factor which, in turn, would be able to activate R. meliloti nifA and fixK.

At least two transcriptional activators which belong to a two-component system, dctD and ntrC, activate genes whose transcription needs an alternative sigma factor, NtrA (10). The upstream regions of these genes contain characteristic NtrA-consensus promoter sequences (10). No such sequences have been identified upstream of R. meliloti nifA or fixK. However, the possibility that fixK and nifA are transcribed by using yet another sigma factor cannot be excluded.

Another point worth mentioning is that although both fixL and fixJ are required for expression of fixK and nifA in R. meliloti, fixJ alone when overexpressed is able to activate the target genes in E. coli. This situation is not unique in the two-component systems. One explanation is that when present at high concentration, one activator protein could be activated by the sensor component of another regulatory couple (16). It will be interesting to see whether one can observe nifA and fixK activation at lower levels of expression of fixJ by introducing fixL on an expression vector in E. coli. This may also give information on the effector to which FixL responds.

We thank J. Prevot for expert secretarial assistance. B. Lesure provided useful advice on the technique of primer extension by reverse transcriptase.

This work was supported by the contract “Fixation biologique de l’azote” (Institut Pasteur, Institut National de la Recherche Agronomique, Société Nationale ELF-Aquitaine, Entreprise minière et chimique) and by a grant from the European Economic Community as part of the Biomolecular Engineering and the Biotechnology Action programmes. C.H. was a recipient of a fellowship from Société Nationale ELF-Aquitaine and Y.R.L. was a recipient of a fellowship from the European Economic Community as part of the Cooperation Programme between the People’s Republic of China and the European Economic Community.

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1738    NOTES


