Binding of activator SyrM to the site of nodD3
P1 region of Rhizobium meliloti *

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Abstract The nodD3 gene of Rhizobium meliloti is transcribed via promotor P1 or P2. Gel retardation assay showed binding of SyrM to the P1 upstream region of nodD3. DNaseI footprint analysis demonstrated that the binding site of SyrM in nodD3 P1 region consists of two inverted repeat sequences arranged in tandem. SyrM seems to bind to DNA in the form of dimer or tetramer and requires the two inverted repeat sequences for binding.

Keywords: DNA footprint, SyrM, nodD3.

The interaction between Rhizobia and their specific host legumes involving the complicated sequential events results in the formation of symbiotic nitrogen fixation nodules[1]. The early event for initiating the nodule formation is governed by the nod genes in Rhizobium. The nod genes are regulated by the regulatory gene nodD. The product of nodD gene, NodD which has common features with the LysR family member acts as transcriptional activator to activate the nod box of the nod genes in the presence of the flavonoid inducers[2,3].

In R. meliloti there are three allelic nodD genes, i.e. nodD1, nodD2 and nodD3. NodD1 and NodD2 activate nod genes in the presence of flavonoid inducers. However, NodD3 activates nod genes independent of inducer[4]. Both the nodD1 and nodD2 genes are constitutively expressed, yet the expression of nodD3 requires the product of the syrM gene, SyrM, also a LysR family protein. The syrM and nodD3 gene are highly homologous and adjacent, but conversely transcribed[4,5]. Since nodD3 expression is regulated by SyrM, and syrM is in turn regulated by NodD3, thus constituting a regulatory circuit[6]. Our previous study has shown that nodD3 is transcribed from two separate promoters P1 and P2. The expression of nodD3 under P1 requires SyrM as well as NodD3, while nodD3 under P2 is expressed feebly and acts only synergically with NodD1 for nod gene activation[7]. Binding of SyrM and NodD3 to the first promoter region of nodD3 has been demonstrated[8]. Recently a putative SyrM binding site was also reported in other laboratory[9]. Little is known about the functional difference between these two promoters of nodD3. Our evolutionary analysis suggests that nodD3 was derived from nodD1 during evolution, with the lately evolved first promoter functioning biologically, and the second one remaining as residue of the original promoter of nodD1[8].

In this paper, we report our recent studies on the binding of SyrM to the first promoter region of nodD3 by DNaseI footprint analysis and the mode of its binding.

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1 Materials and methods

1.1 Strains and plasmids

*R. meliloti* strain 1021 is a streptomycin-resistant (str) derivative of wild type strain SU47. Strain JM57 carries the nod-lacZ fusion integrated to the symbiotic plasmid pSym of *R. m1021*. R.mD1D2-3, a double mutant containing nodD1: Tn5 (9B7) and nodD2: tm in Rm1021\[10\]. pMH904 was constructed by cloning the 4.2 kb HindIII-ClaI fragment containing the syrM gene into pWB5\(_A\)(Tc\(^\+)

1.2 Protein partial purification by ammonium sulfate precipitation

Cell extract was prepared essentially according to Hong et al. The *Rhizobium* culture grown to late-log phase was harvested by centrifugation, then the pellet was suspended in TES buffer (25 mmol/L Tris-HCl, pH 7.5, 5 mmol/L EDTA, 5 mmol β-Mercaptoethanol, 150 mmol/L sodium chloride, 10% (v/v) Glycerol). Cells were lysed by sonication, and the debris were removed by centrifugation. Protein was fractionated by adding saturated ammonia sulfate to the supernatant with constant stirring at 0°C. The pellet was collected by centrifugation and then resuspended in 2 x vol TES buffer. After dialysis to TES buffer overnight, the protein preparation was frozen to −70°C prior to experiment. When the extract was subjected to ammonia sulfate fractionation, SyrM was precipitated mainly at concentration between 35%–40% (SyrM activity was monitored by specific gel retardation assay used as follows) and NodD3 was precipitated mostly at concentration of 45%\[12\].

1.3 Gel retardation assay

Gel retardation was performed majorly according to Fried and Crothers\[13\]. In 1 x binding buffer (50 mmol/L Tris-HCl, 100 mmol/L sodium chloride, 2 mmol/L magnesium chloride, 0.1 mmol/L dithiothreitol, 10% glycerol) were mixed 20 000 cpm α-dATP\(\text{\textsuperscript{32}P}\)-end-labeled DNA probe, adequate amount protein and carrier DNA 2 μg (calf thymus DNA) in a total volume of 20 μL. After incubation for 20 min at room temperature, the samples were resolved on native polyacryamide gel (5%).

1.4 DNaseI footprint analysis

DNaseI footprinting experiments were carried out with the 300 bp EcoRI-HindIII (label the template strand at EcoRI site and the non-template strand at HindIII site with \textsuperscript{32}P-dATP) and the partially purified protein preparation of JM57/pMH904. A total of 2 x 10\(^6\) cpm DNA probe mixed with different amounts of SyrM protein and 2 μg carrier DNA in a final volume of 20 μL binding buffer was incubated for 30 min at 28°C. After addition of adequate amount of DNaseI, the reaction mixture was incubated for another 1 min at 30°C. It was sequenched by adding 200 μL stopping buffer (25 mmol/L EDTA, 10 μg yeast tRNA/300 mmol/L sodium acetate), and precipitated by adding ethanol at −70°C for 5 min. The sample was resuspended in 10 μL formamide loading buffer and was analyzed in denaturing 8% polyacrylamide gel.

1.5 Labeling of double-strand oligonucleotide fragments

The sequence of the synthetic oligonucleotides was presented as follows: for ISR1: 1, 5’ TAGTCTTCAACCATACCCAGCGGATGATGG 3’