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Corynebacterium ammoniagenes RNA Polymerase Genes : Genetic Information and Its Implication on Metagenome Research

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Abstract

Corynebacteria are evolutionally located at low GC group of eubacteria and have been known for important industrial strains for the production of nucleic acid or amino acid, however, studies on transcription, regulation mechanisms and genome information are still limited. In order to understand transcription process and its regulations of Corynebacteria, we initiated cloning of the genes encoding RNA polymerase(RNAP) from *Corynebacterium ammoniagens* and investigated its function to gain insights on heterologous genome information utilization.

The *rpo* A gene(α subunit of RNAP) was made of 1,005 base pairs encoding 334 amino acid with a calculated molecular mass of 37 kDa. Expression of His-tagged Cam-α in *E. coli* indicated the predicted molecular weight. Sequence analysis of the region surrounding *rpoA* identified six open reading frames that are found in the conserved gene order of IF-1, S13, S11, S4, RNAPα and L17 found in the α-proteobacteria. The C-terminal domain of α(α-CTD) contained F262, R265, N268, C269, G296, K298, and S299 residues which have been known important for DNA binding and UP element function. *C. ammoniagenes* RNAP α exhibited 82%, 70% and 42% similarity to *C. glutamicum, M. tuberculosis,* and *E. coli,* respectively. To find the transcription initiation(+1) site, we tested primer extension experiment with three different of oligonucleotide primers which are S13 N-terminal region sequences. Transcription start site located far from the upstream of S13. It was different from *E. coli* α operon.

The *rpo*B *and rpo*C(*β*, β' subunit of RNAP)are involved in most of the catalytic functions of RNAP, including nucleotide binding, transcription initiation, elongation, termination, and interaction with both the sigma subunit and the NusA. We cloned a 11,433 bp fragment and this fragment contained the genes for hypothetical membrane protein, oxidoreductase, hypothetical protein, RNAP β(*rpo*B), β' (*rpo*C). This gene organization is quite different from that found in other bacteria even from closely related strain *C. glutamicum*. The *rpoB* consisted of 3,492 bp encoding 1,164 amino acid of 129.3 kDa and *rpo*C consisted of 4,011bp encoding 1,337 amino acid of 155 kDa. S1 mapping and primer extension analysis revealed that the *rpo*B and *rpo*C gene exist as an operon and +1 site was located upstream of *rpoB*. There were no typical *E. coli* sigma

70 type promoter sequences in promoter region identified experimentally.

The *rpo*D gene(sigma subunit of RNAP) was consists of 1,404 base pairs encoding 468 amino acid with a calculated molecular mass of 52kDa. RpoD revealed that *C. ammoniagenes* has four highly conserved domain typical for major sigma factor of eubacteria as well as Lys rich region in N-terminal part. Northern analysis indicated that the *rpo*D gene was transcribed during all growth stage with diminished extent or more vulnerable to degradation in stationary phase, and monocistron judged by the size of transcript. Primer extension revealed characterisitic promoter region different from *E. coli* consensus type.

The α-CTD of the *E coli* RNAP contains the DNA binding determinants responsible for interactions with UP elements. Genetic analyses have identified amino acid residues in the CTD critical for DNA binding and UP element function. These residues are essential for cell viability in *E. coli* and are nearly invariant in bacteria. However it is not known whether it binds to DNA in all bacteria. We tested whether *C. ammoniagenes* α could bind *E. coli* UP element DNA fragments with electrophoretic mobility shift assays. *C. ammoniagenes* α did not shift *E. coli* proximal, or full consensus UP element oligonucleotides, while *E. coli* α shifted them efficiently under the condition we used. To evaluate in vivo functions, we tried complementation experiments using the *E. coli* HN317 strain which is a *rpoA*^{ts} mutant at the nonpermissive temperature. *C. ammoniagenes* α did not restored viability to the *rpoA*^{ts} mutant at the nonpermissive temperature. It's complementation ability was similar to the *E. coli* R265A mutant which is defective in UP elememt activation. Therefore purified Cα could not interact with *E. coli* UP element in vitro and could not replace function of Eα in vivo. However, reconstituted RNAP holoenzyme from Cα and *E. coli* β, β', and sigma 70 subunits interacted very efficiently with *E. coli* UP element by increasing promoter strength. This fact suggests that α-CTD of *C. ammoniagenes* is functioning properly when assembled with the other parts of RNAP from *E. coli*.

These findings have important indications on metagenome research of gram positive bacteria and may be of use for biotechnological applications.

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