Vol. 121, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 31, 1984 Pages 41-46

NUCLEOTIDE SEQUENCE OF THE PROMOTER REGION OF THE MELIBIOSE OPERON OF *Escherichia coli*

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Received April 16, 1984

Summary: The nucleotide sequence of the promoter region of the melibiose operon of *E. coli* was determined. Consensus sequences for the -35 region, the Pribnow box and the binding site for cyclic AMP receptor protein were found in this region. The possible secondary structure of this DNA region was very similar to that of the promoter region of the lactose operon. A possible initiation ATG preceded by a Shine-Dalgarno sequence with proper spacing was present just downstream of the promoter region. The possible sequence of 52 amino acid residues in the NH_2 terminus of the α-galactosidase were determined.

The melibiose operon of *E. coli* is located at about 93 min on the genetic map (1). This operon contains structural genes for α-galactosidase (*melA*) and the melibiose carrier (*melB*) (2). The gene composition of this operon is similar to that of the lactose operon which contains genes for β-galactosidase (*lacZ*) and the lactose carrier (*lacY*) (3). The properties of the melibiose operon are also similar to those of the lactose operon. These operons are both inducible (3,4), and are under the control of catabolite repression (3,5).

Previously we reported construction of recombinant plasmids carrying various portions of the melibiose operon (6), and we determined the organization of the melibiose operon to be promoter-*melA*-*melB* (6). Sequence analyses of the 3'-flanking region of the *melB* gene suggested the presence of a third structural gene downstream of *melB* (7). Since there is a third gene in the lactose operon that codes for transacetylase, the putative third gene (tentatively designated as *melC*) in the melibiose operon might code for a

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Abbreviations: CRP, cyclic AMP receptor protein.

0006-291X/84 $1.50

similar enzyme. From genetic and biochemical studies, we estimated the locations of the promoter and melAB on the DNA fragment (6,7).

In the present study, we determined the nucleotide sequence of the promoter region of the melibiose operon encompassing the first portion of the melA gene. We also examined the structural similarities of these regions to those of the lactose operon.

MATERIALS AND METHODS

Bacteria and Plasmids E. coli strain RA11 (8), a derivative of K12, was used for plasmid propagation. The plasmid used for isolation of the DNA fragment was pSTY81-30 (6) carrying the EcoRI-BamHI fragment of the melibiose operon cloned in pBR322 (Fig. 1). Cells of RA11/pSTY81-30 were grown in L broth (9).

Preparation of DNA Plasmid DNA was amplified in the presence of chloramphenicol (10) and prepared by a published procedure (11). DNA fragments digested with restriction endonucleases were separated by polyacrylamide gel electrophoresis and eluted from the gel (12).

Nucleotide Sequence DNA fragments were 5' labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (13). The nucleotide sequence was determined by the method of Maxam and Gilbert (12).

RESULTS AND DISCUSSION

Nucleotide Sequence Previously we determined the organization of the melibiose operon to be promoter, melA and melB in this order (Fig. 1) (6). Furthermore, we found that the promoter was located on the left-hand side of the PstI site, while functional melA was located on the right-hand side of

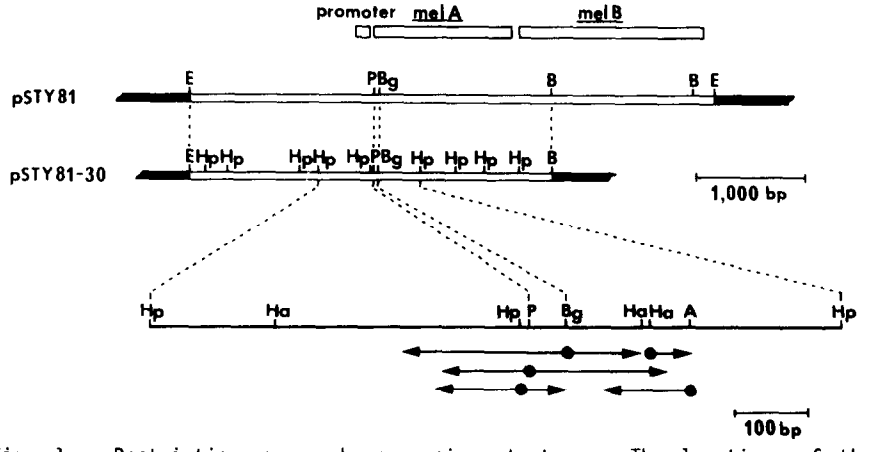

Fig. 1. Restriction map and sequencing strategy. The locations of the promoter, melA and melB on the plasmid pSTY81 are shown at the top. Regions shown as solid bars are derived from vector pBR322. The lower portion shows the sequencing strategy. Arrows indicate the directions and extents of sequences obtained from the labeled end of each fragment. The sites of action of restriction enzymes are as follows: E, EcoRI; P, PstI; Bg, BglII; B, BamHI; Hp, HpaII; Ha, HhaI; A, AluI. bp, base pair.

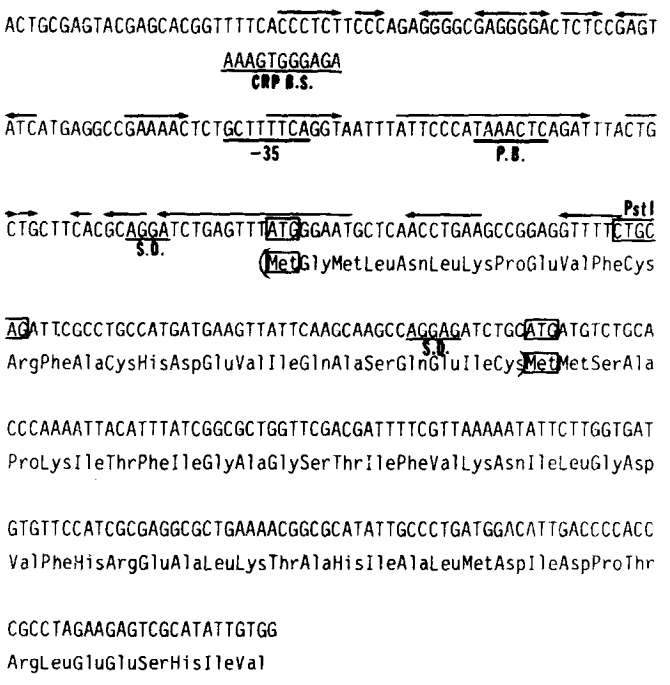

Fig. 2. Nucleotide sequence of the promoter region of the melibiose operon. The -35 region (-35), Pribnow box (P.B.), Shine-Dalgarno sequences (S.D.) preceding ATG, and the PstI site are indicated. A possible CRP binding sequence (complementary strand) (CRP B.S.) is also shown. Dyad symmetries are indicated by arrows. The possible amino acid sequence of the NH_2 terminus of α-galactosidase is also shown.

the PstI site (6). Thus the promoter seemed to be located close to the PstI site. We determined the nucleotide sequence of a DNA segment of about 380-bases long covering the regions upstream and downstream of the PstI site (Fig. 2). Consensus sequences for the -35 region (14) and the Pribnow box (15) were present in this region. Although the nucleotide sequence of the -35 region (GCTTTTCA) was not very typical, it was very similar to that of the -35 region of the lactose promoter (GCTTTACA) (14).

Since expression of the melibiose operon is under the control of catabolite repression (5), a consensus sequence for the binding site of CRP was expected to be present upstream of the -35 region. The consensus sequence for CRP binding proposed by O'Neill et al. (16) is shown in Fig. 3. A sequence which is very similar to the complementary sequence of the consensus sequence is present about 50 nucleotides upstream of the -35 region (Figs. 2 and 3). The structure of double stranded DNA in this region must be

consensus sequence	5'—AAAGTGTGACA—3'
mel	5'—AGAGGGTGAAA—3'
lac 1	5'—TTAATGTGAGT—3'
lac 2	5'—AATGAGTGAGC—3'
gal	5'—AAAGTGTGACA—3'
araBAD	5'—AAAGTGTGACG—3'
araC	5'—AAAGTGTCTAT—3'

Fig. 3. Alignment of possible CRP binding sites. A possible CRP binding sequence (complementary strand) of the melibiose promoter is aligned for homology with other promoter CRP binding sites. Identical nucleotides to the consensus sequence are marked. Sequences other than that of the melibiose system are cited from ref. 16.

very similar to that of the binding site for CRP proposed by O'Neill et al. (16). The possible secondary structure of this region supports the view that this region is the binding site for CRP, as shown below.

Possible Secondary Structure The possible secondary structure of the promoter region of the melibiose operon is shown in Fig. 4, in comparison

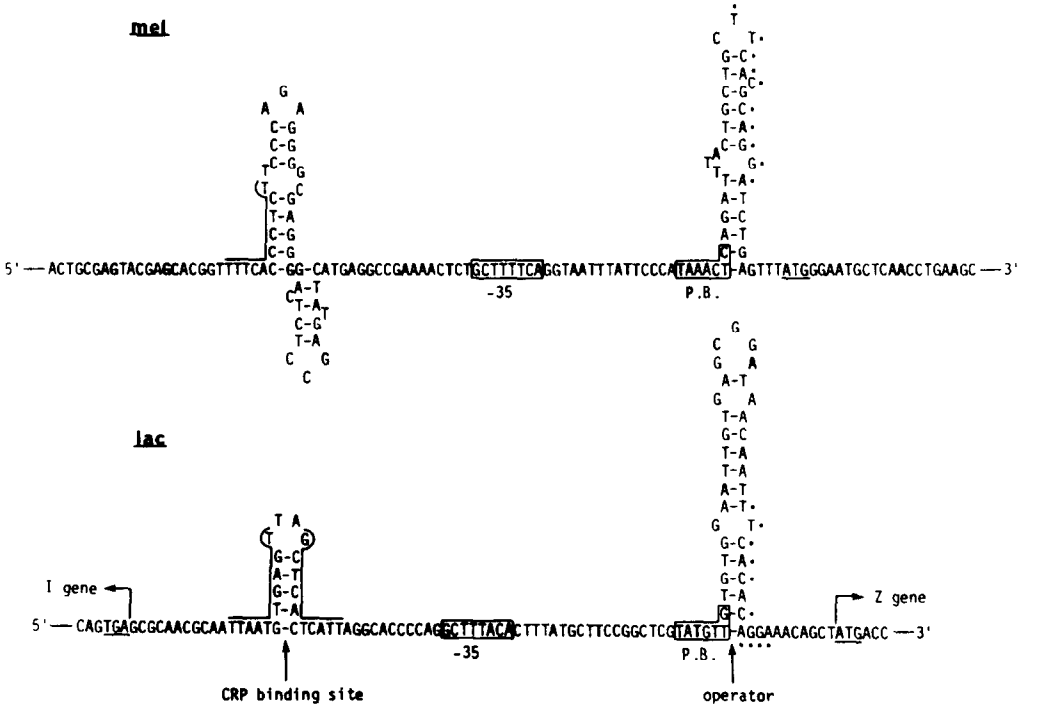

Fig. 4. Possible secondary structures. Possible stem-loop structures found in the melibiose promoter region are shown in comparison with those of the lactose promoter region. The reported CRP binding site and operator of the lactose system are indicated. Consensus sequences for the CRP binding site are indicated by lines. The -35 region (-35) and Pribnow box (P.B.) are boxed. Homologous sequences found in stem-loop structures are indicated by dots.

with that of the lactose operon. The Pribnow box of the melibiose promoter is followed by a stem-loop structure. A very similar structure has been reported in the lactose operator (17,18). Almost identical sequences (10 of 11 nucleotides are identical) were found in the stem-loop structures (Fig. 4). Therefore the stem loop structure present following the Pribnow box of the melibiose promoter seems very likely to be the operator, the binding site for repressor. It is reasonable to suppose that the melibiose repressor, if present, and the lactose repressor are structurally similar to each other. The fact that melibiose is a common inducer for the expressions of both operons (3,4) supports this view. It should be noted that a longer stem-loop could be formed in the melibiose promoter region, as indicated by the arrows in Fig. 2.

As mentioned in the preceding section, a probable region for the binding of CRP was found upstream of the -35 region. This region can form a stem-loop (Fig. 4). A structural characteristic of the binding site for CRP in the lactose promoter region is a stem-loop form (19). Therefore, the stem-loop structure located upstream of the -35 region seems to be the binding site for CRP.

Deduced Amino Acid Sequence of the NH_2 Terminus of α-Galactosidase As we reported previously (6), functional melA is located downstream of the PstI site (Fig. 1). We have identified the melA gene product as a protein with an apparent molecular weight of 50,000 daltons (6). We have also determined the whole nucleotide sequence of the melB gene (7). Judging from the size of the melA gene product and the location of the melB gene on the DNA segment, melA seems to start immediately downstream of the PstI site.

A Shine-Dalgarno sequence (20) (AGGAG) followed by ATG with a spacing of 6 nucleotides was found just downstream of the PstI site (Fig. 2). In the nucleotide sequence determined the frame starting with ATG is not interrupted by a termination codon. It seems highly likely that this ATG is the initiation site for α-galactosidase. Thus α-galactosidase seems to start with Met followed by Met, Ser, Ala, Pro, and other amino acids, although it

is not known whether processing of the polypeptide occurs. On the other hand, another Shine-Dalgarno sequence (AGGA) followed by ATG with a 9 nucleotide spacing was found just upstream of the PstI site (Fig. 2). The frame starting from this ATG was the same as that described above. But this ATG is less likely to be the initiation site for α-galactosidase, because a spacing of more than 9 nucleotides has been reported to be rare (21). However, if this ATG can function as the initiation site, the first 28 amino acid residues shown in Fig. 2 (in parentheses) are not necessary for the function of α-galactosidase.

Acknowledgments This research was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture of Japan. We thank Dr. Hiroshi Kanazawa for valuable suggestions.

REFERENCES

1. Backmann, B. J. (1983) Microbiol. Rev. 47, 180-230.
2. Schmitt, R. (1968) J. Bacteriol. 96, 462-471.
3. Beckwith, J. R. (1970) The Lactose Operon, pp. 27-47, Cold Spring Harbor Laboratory, Cold Spring Harbor.
4. Prestidge, L. S., and Pardee, A. B. (1965) Biochim. Biophys. Acta 100, 591-593.
5. Okada, T., Ueyama, K., Niiya, S., Kanazawa, H., Futai, M., and Tsuchiya, T. (1981) J. Bacteriol. 146, 1030-1037.
6. Hanatani, M., Yazyu, H., Shiota-Niiya, S., Moriyama, Y., Kanazawa, H., Futai, M., and Tsuchiya, T. (1984) J. Biol. Chem. 259, 1807-1812.
7. Yazyu, H., Shiota-Niiya, S., Shimamoto, T., Kanazawa, H., Futai, M., and Tsuchiya, T. (1984) J. Biol. Chem. in press.
8. Lopilato, J., Tsuchiya, T., and Wilson, T. H. (1978) J. Bacteriol. 134, 147-156.
9. Lennox, E. S. (1955) Virology 1, 190-206.
10. Clewell, D. B. (1972) J. Bacteriol. 110, 667-676.
11. Meyers, J. A., Sanchez, D., Elwell, L. P., and Falkow, S. (1976) J. Bacteriol. 127, 1529-1537.
12. Maxam, A. M., and Gilbert, W. (1980) Methods Enzymol. 65, 499-560.
13. Walseth, A. M., and Johnson, R. A. (1979) Biochim. Biophys. Acta 526, 11-31.
14. Rosenberg, M., and Court, D. (1979) Ann. Rev. Genet. 13, 319-353.
15. Pribnow, D. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 784-788.
16. O'Neill, M. C., Amass, K., and Crombrugghe, B. D. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 2213-2217.
17. Gilbert, W., and Maxam, A. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 3581-3584.
18. Dickson, R. C., Abelson, J., Barnes, W. M., and Reznikoff, W. S. (1975) Science 187, 27-35.
19. Reznikoff, W. S., and Abelson, J. N. (1978) The Operon, pp. 211-243. Cold Spring Harbor Laboratory, Cold Spring Harbor.
20. Shine, J., and Dalgarno, L. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 1342-1346.
21. Gold, L., Pribnow, D., Schneider, T., Shinedlind, S., Singer, B. S., and Stormo, G. (1981) Ann. Rev. Microbiol. 35, 365-403.