Volume 9 Number 9 1981 Nucleic Acids Research

Identification, nucleotide sequence and expression of the regulatory region of the histidine operon of Escherichia coli K-12

Pasquale Verde*, Rodolfo Frunzio, Pier Paolo di Nocera, Francesco Blasi* and Carmelo B.Bruni

Centro di Endocrinologia ed Oncologia Sperimentale del C.N.R. and Cattedra di Microbiologia, Istituto di Patologia Generale, University of Naples, 2nd Medical School, Via S. Pansini 5, 80131 Naples, Italy

Received 12 February 1981

ABSTRACT

A restriction fragment has been isolated and its nucleotide sequence determined. This fragment contains sites for RNA polymerase binding, initiation and termination of transcription of the Escherichia coli histidine operon. In vitro transcription of plasmids containing this region generates one single histidine-specific, attenuated, small RNA: the leader RNA. This RNA is more efficiently transcribed when the template DNA is supercoiled.

Another promoter was identified on the same fragment of deoxyribonucleic acid by in vitro transcription, DNA sequencing and RNA polymerase binding. Both promoters, transcribing in opposite direction, are very A-T rich and are separated by a G-C rich region containing a palyndromic structure.

INTRODUCTION

The histidine operon, like several other biosynthetic operons in bacteria, is regulated at the level of transcription via the attenuation mechanism (1). The RNA molecules, initiated at the promoter site, have two alternatives: they can either elongate into the structural genes or terminate at the attenuator site. This mechanism is in turn regulated by translation of a leader peptide sequence present on the leader RNA, rich in histidine codons (2, 3). The level of charged tRNA controls the rate of translation of the peptide, i.e. the flow of ribosomes on the leader RNA. Ribosomes, in turn, can affect the secondary structure of the leader RNA. If the attenuator stem and loop structure is formed, termination ensues and the operon is repressed. If ribosomes are stalled on the leader RNA at specific codons, because of a limitation of histidyl tRNA, an alternative structure may form, which prevents formation of the attenuator. In this case the RNA polymerase can transcribe beyond the attenuator barrier into the structural genes and the operon is derepressed (1, 4, 5).

We have studied the expression and organization of the his operon of E. coli K-12 and have previously reported the cloning of a HindIII DNA fragment containing the proximal part of the operon (6). The fragment of DNA contain-

ing the first structural gene was identified by subcloning (2). Nucleotide sequencing of the region upstream of the first structural gene identified the attenuator and suggested the existence of a leader region which is transcribed into a his leader RNA (2). A restriction map of the entire region was reported (7).

On the basis of this information we now report the identification and isolation of the E. coli his operon regulatory region, its complete nucleotide sequence and its functional characterization.

MATERIALS AND METHODS

Chemicals and enzymes. Restriction enzymes, ApyI, AluI, HhaI, HinfI, HapII and HindIII were a gift from R. Di Lauro. BglII was purified as described (8), TaqI and BamHI were from Miles Research Laboratories, PstI and EcoRI were from Bethesda Research Laboratories. RNA polymerase was purchased from Boehringer, Mannheim, or from Miles. T4 polynucleotide kinase and T4 DNA ligase were from Bethesda Research Laboratories. DNA polymerase (Klenow) fragment) was from Boehringer. γ-^{32}P ATP was from Amersham (2,000-3,000 Ci/mmole) or was prepared as described (9) with a specific activity of about 2,000 Ci/mmole. Inorganic ^{32}P carrier-free, α-^{32}P ribonucleoside triphosphates (350-400 Ci/mmole) and α-^{32}P deoxyribonucleoside triphosphates (350-400 Ci/mmole) were from Amersham.

RNA polymerase binding to DNA. Purified HinfI 730 bp[§] fragment (see below) was digested with restriction endonuclease ApyI or double-digested with ApyI and BglII. The reaction mixtures were phenol-extracted, phenol removed with 5-6 extractions with ether, the DNA precipitated with ethanol and resuspended in 10 mM Tris-HCl, pH 7.9, 1 mM EDTA[§]. RNA polymerase binding to DNA fragments was performed according to the procedure of Jones and Reznikoff (10). DNA fragments retained on filters were eluted (10) and run on a 7% acrylamide slab gel, stained with ethidium bromide and photographed with a Polaroid camera under UV lights.

Isolation of DNA and purification of DNA fragments. Total E. coli DNA was prepared according to Thomas et al. (11). Plasmid DNA was prepared by isopicnic ultracentrifugation in cesium chloride-ethidium bromide (12).

The 950 bp BglII D fragment (6), used to construct plasmid pPV1 (see below), was purified on a 5-20% sucrose gradient of a BglII digest of plasmid pCB3 (7). The 730 bp HinfI fragment was isolated from a total HinfI digest of plasmid pCB3 DNA run on a 6% acrylamide slab gel. The band was excised from the gel and the DNA eluted as described (13).

Plasmid construction and transformation. Plasmids pBR322 and pBR313 (14), pCB3 (7) and pCB5 (2) have already been described. Plasmid pPVI1 was derived by inserting the purified 950 bp BglII D fragment into pBR322 at the single BamHI site (2). The DNA was ligated, redigested with BamHI, to eliminate non-recombinant pBR322 molecules (2), and used to transform (15) strain FB190, hisG2743 recA56 (7), selecting for ampicillin resistance and scoring for tetracycline sensitivity. Plasmids pPV3 and pPV4 were obtained by ligating HindIII-restricted total E. coli DNA from wild type strains UTH1038 and UTH653 (16) to HindIII-linearized pBR322 DNA. his auxotrophic strain FB190 was transformed selecting for growth in minimal medium and resistance to ampicillin.

In vitro transcription. The reaction mixtures (50 µl) contained: 1 pmole of DNA, 20 mM Tris-HCl, pH 7.9, 100 mM KCl, 10 mM $MgCl_2$, 0.1 mM dithiothreitol, 1 mM EDTA and RNA polymerase (50-100 µg/ml). After 10 min at 37°C heparin was added at a final concentration of 100 µg/ml, followed, after 1 min, by nucleotide triphosphate (final concentration 200 µM). The labeled triphosphate was at a concentration of 40 µM. Each reaction contained 20-40 µCi of an α-^{32}P triphosphate (generally GTP). After 30 min at 37°C reactions were stopped with 250 µl of a solution containing 0.1% SDS[§], 10 mM EDTA and 10 mM Tris-HCl, pH 8.0. This mixture was extracted with phenol and precipitated with ethanol. Pellets were resuspended in deionized formamide containing 0.05% bromophenol blue and 0.05% xylene cyanol. After 15 min at 37°C the samples were heated at 80°C for 2 min, chilled on ice and loaded on a 0.4 mm thick 6% acrylamide slab gel in 7 M urea. Electrophoresis was run at 30 watts. Slabs were then autoradiographed on Kodak X-Omat R X-ray film.

DNA sequencing. DNA sequences were determined according to the technique of Maxam and Gilbert (13). Thin gels were prepared according to Sanger and Coulson (17). Procedures for the 5' and 3' labeling of the DNA are described (13). Sequencing strategy is reported in the Results section.

RESULTS

From the data on the structure of the his operon of E. coli K-12 (2, 6, 7) we were able to identify a 730 bp HinfI fragment which should contain all the regulatory region of the his operon including the promoter site. This fragment has been recognized because it contains a single BglII site (2, 6). The DNA of plasmid pCB3 has been digested with HinfI or double-digested with HinfI and BglII and run onto a 6% acrylamide slab gel (Fig. 1). The fragment was subsequently purified from the gel (see Methods).

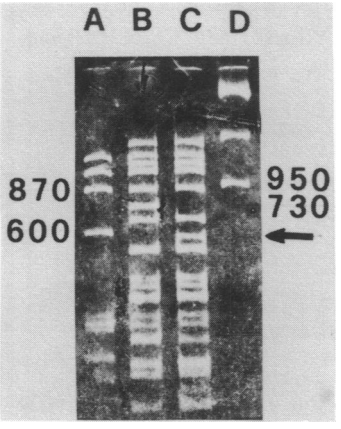

Fig. 1. 6% acrylamide slab gel electrophoresis of plasmid pCB3 DNA digested with HinfI, BglII or with both restriction enzymes. Lane A: Φ X174 DNA digested with HaeIII and used as marker. The 870 and 600 bp fragments are indicated. Lane B: pCB3 digested with HinfI, the 730 bp band is present. Lane C: pCB3 digested with HinfI and BglII, the 730 bp band is absent, while two new bands are generated; one 560 bp (arrow) and another one 170 bp (not visible). Lane D: pCB3 DNA digested with BglII. The 950 bp band, cloned in pPV1, is indicated.

Identification of the RNA polymerase binding sites. In order to verify that this fragment actually contains the his promoter, we have performed binding studies with the E. coli RNA polymerase. The results are reported in Fig. 2. The DNA-protein complexes, bound to nitrocellulose filters, have been eluted and electrophoresed on a 7% acrylamide gel along with appropriate controls.

Figure 2, lane B, shows that RNA polymerase does bind to the undigested HinfI fragment. Lane E shows that both fragments generated by digestion with ApyI are bound to RNA polymerase (see bottom part of Fig. 2 for a restriction map of the HinfI fragment). When the DNA is further digested with BglII, generating now three fragments (Fig. 2, lane I), only two of them bind RNA poly-

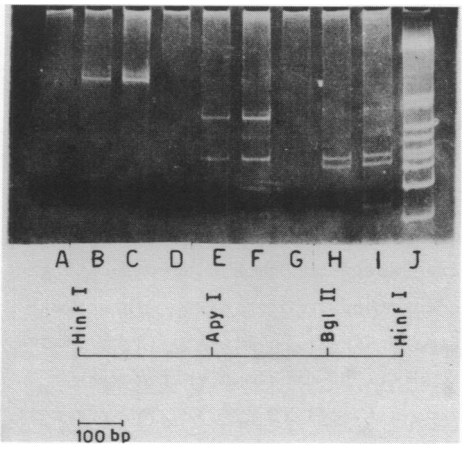

Fig. 2. 7% acrylamide slab gel of DNA fragments bound by RNA polymerase. Lanes A, D and G: controls without polymerase. Lanes C, F and I: input DNA (1 µg) of HinfI 730 bp fragment (C), digested with ApyI (F), and with ApyI and BglII (I). Lanes B, E and H: DNA fragments bound to polymerase and retained on the filters. HinfI 730 bp (B), ApyI 430 and 300 bp (E), ApyI and BglII 300 and 260 bp (H). The BglII-HinfI 170 bp fragment is not retained. Lane J: SV40 DNA digested with HinfI and used as a marker. The bottom part of the Figure shows the relevant restriction sites on the HinfI 730 bp fragment.

merase and are retained on the filter (Fig. 2, lane H). These results suggest that there are two different binding sites for RNA polymerase, rather than just one. Of the two ApyI fragments that bind RNA polymerase, the 430 bp fragment covers the attenuator region of the his operon, previously sequenced (2), and should, therefore, contain the his promoter. The data of Fig. 2 demonstrate that this binding site is located between the ApyI and the BglII sites, i.e. upstream of the attenuator which is located between BglII and HinfI (2).

The presence of a second RNA polymerase binding site was unexpected and required further investigation. The purified 730 bp HinfI fragment was transcribed in vitro. In agreement with the RNA polymerase binding experiment, two transcripts were observed. One represented the his leader RNA and the other, named "A" RNA, was a run-off RNA, 230 nucleotides long, transcribed in a direction opposite to that of the his leader RNA. The 5' termini of the two transcripts were sequenced. These data have been reported elsewhere (18).

In vitro transcription. In vitro synthesis of the his leader RNA was studied using entire supercoiled plasmids DNA as templates. Three different plasmids were used in order to verify that transcription was independent of: a) orientation of the his genes on the plasmid (pCB3 and pPV4); b) source of the inserted DNA (pCB3, pPV3 and pPV4); c) size of the insert (pPV3 and pPV4). All three plasmids (Table) contained the first two structural genes and the his regulatory region on a HindIII 5,300 bp fragment. Plasmid pCB3 (7) is a pBR313 (14) derivative in which the inserted 5,300 bp fragment was cloned from a his transducing phage λhisG (19).

TABLE

Plasmids used as templates for in vitro transcription

Plasmid	Source of cloned DNA	Size of insert	Orientation of the 5,300 bp insert	References
pPVI	pCB3	950 bp	---	6, 7, this paper
pCB3	λhisG	5,300 bp	parallel[a]	7, 21
pPV3	UTH 1038	5,300 bp	not known	16, this paper
pPV4	UTH 653	9,300 bp[b]	anti-parallel[a]	16, this paper

(a) Parallel orientation means: HindIII hisOGD HindIII; anti-parallel orientation: HindIII hisDGO HindIII (see Ref. 7 for detailed restriction map).
(b) The 9,300 bp insert arises from cloning of a partial HindIII digest, and is made up of two fragments: one of 5,300 bp and the other of 4,000 bp.

The products of in vitro transcription were extracted and run on a 6% acrylamide slab gel under denaturing conditions. Figure 3 (Panel I) shows the autoradiogram of the gel. Lanes A, C and D are the RNA transcribed from the three different plasmids, pCB3, pPV3 and pPV4, respectively. In all cases two major bands appear, one about 100 (R) and the other about 180 (L) nucleotides long. The first band is possibly homologous to the 104 bases long RNA of ColE1 (20) transcribed from a very strong promoter close to the plasmid origin of replication (21). The second band has the right size to be the leader RNA of the his operon. In a different paper we have demonstrated that the L RNA is in fact the his leader RNA, initiated at the his promoter and terminated at the end of the run of T's of the attenuator (18). This band is missing when plasmid pPV1 DNA was used as template. In this plasmid the regulatory region of the his operon, deleted of the attenuator (2), is contained in a 950 bp BglII (Fig. 1 D) which has been recombined into the BamH1 site of pBR322 (see Methods). As expected, the his leader RNA is not synthesized (Fig. 3, Panel I, lane B). The band is also missing when the DNA of the plasmid vectors pBR322 and pBR313 was used as template (Fig. 3, Panel I, lanes E and F).

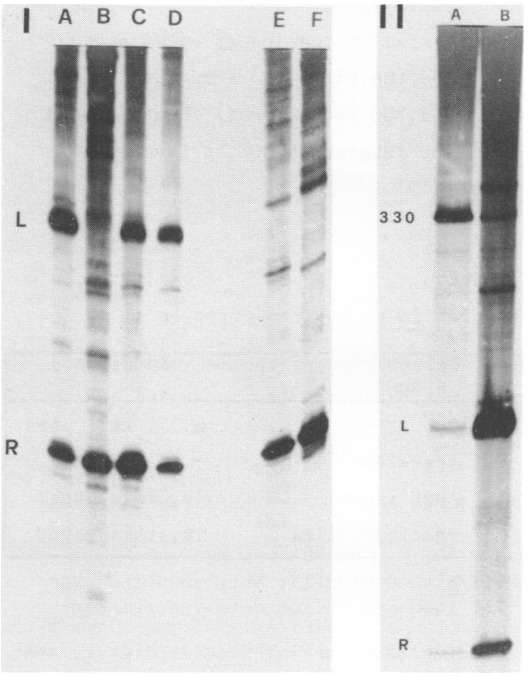

Fig. 3. 6% acrylamide slab gel electrophoresis of in vitro transcription of plasmids DNA. Panel I. Lane A: RNA synthesized with plasmid pCB3 as template; lane B: plasmid pPV1; lane C: plasmid pPV3; lane D: plasmid pPV4; lane E: plasmid pBR322; lane F: plasmid pBR313. Panel II. RNA synthesized from linear plasmid DNA. Lane A: linear pCB3 DNA template restricted with EcoRI; lane B: supercoiled plasmid pCB3 DNA. R, 100 bases RNA; L, his leader RNA; 330, transcript of unknown origin. See Results and Methods for further details.

We have investigated the effect of the supercoiled structure of the template on the efficiency of transcription of the his leader RNA (Fig. 3, Panel II). The RNA synthesized by equal amounts of supercoiled or EcoRI linearized plasmid pCB3 DNA was electrophoresed and autoradiographed. The individual bands were excised and counted. The his leader RNA (L) was transcribed 18 fold more efficiently when the DNA template was in the supercoiled form. The 100 nucleotides long RNA (R) was transcribed 10 fold more efficiently from the supercoiled template, as already reported by others for the 104 nucleotides long RNA of ColE1 (20). Another transcript of unknown origin, approximately 330 nucleotides long is also present, but its rate of transcription was not affected by linearization of the template.

DNA sequence of the hinfI 730 bp fragment. Part of the sequence around the BglII site was reported earlier and allowed identification of the attenuator region of the E. coli his operon (2). We have now completed the DNA sequence of the HinfI 730 bp fragment. Figure 4 shows the strategy adopted in sequencing the fragment.

The region covering the two transcription initiation sites have been sequenced on both strands, either by using different fragments labeled at the 5' end with polynucleotide kinase, or by using the same fragment labeled in two different ways: in one case at the 3' end with Klenow DNA polymerase, in the other at the 5' end with kinase. Other parts of the sequence have been confirmed sequencing the same strand, although on different fragments.

Figure 5 reports the DNA sequence of the fragment. The two arrows at nucleotides 222 and 447 indicate the 5' ends of the "A" and his leader RNA, respectively (18). Boxed sequences represent Pribnow boxes or -35 TTG recognition sequences (22). The underlined sequence from nucleotide 325 to 347 represents

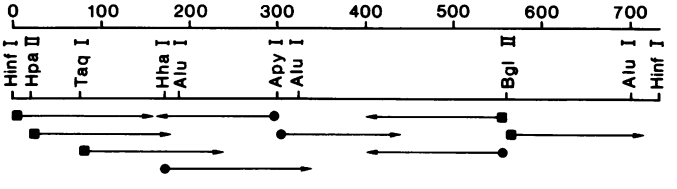

Fig. 4. Strategy for sequencing the 730 bp HinfI fragment. Numbers on top are bp. Only the restriction sites used for sequencing are shown. The horizontal arrows indicate the direction and the range of the sequence. Closed squares at the beginning of the arrows indicate that the fragment was labeled at the 5' terminus using T4 polynucleotide kinase and γ-^{32}P ATP. Closed circles indicate that the fragment was labeled at the 3' terminus using Klenow polymerase and α-^{32}P dN§TP.

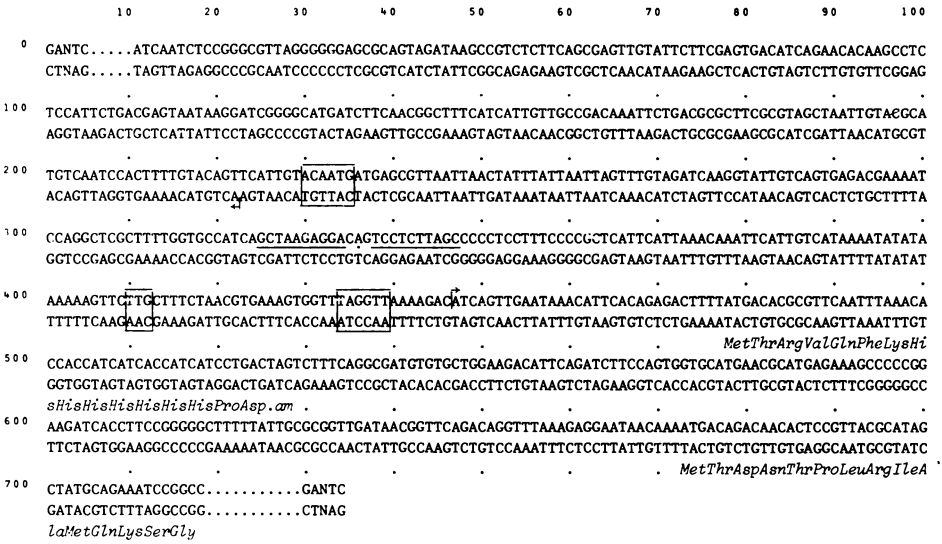

Fig. 5. Nucleotide sequence of the E. coli K-12 his operon regulatory region. Nucleotide sequence of both strands is displayed. For further details see the Results and Discussion sections.

a palyndrome. Palyndromes present in the his leader RNA region are not indicated. They have been discussed elsewhere (1). The amino acids sequence of the leader peptide (nucleotide 478-528) and of the N-terminal region of the product of the first structural gene hisG, ATP-phosphoribosyl transferase (nucleotides 673-717) are reported.

Part of the sequence from nucleotide 478 to 675 was previously published (2). The following corrections need to be made: nucleotide at position 556 is A and not, as previously reported, G; the sequence 623-627 is TTATT and not ATATAATTA; nucleotides 658, 662, 668, 671 and 672 were missing.

DISCUSSION

Binding of purified RNA polymerase to purified DNA fragments, in vitro transcription of plasmid DNA and of purified fragments, and DNA sequencing data have allowed us to identify and define the promoter of the his operon of E. coli K-12. We have also identified a second RNA polymerase binding site close to the his promoter. The his promoter is used as a transcription initiation site for the his leader RNA both in vitro and in vivo (18). This RNA is 180 nucleotides long and is terminated at the attenuator site at position

626 (18, see Fig. 5). In vitro transcription of plasmids DNA yields the his leader RNA regardless of the orientation of the his genes in the vector DNA or of the size or source of the inserted piece (Fig. 3, Panel I).

A 104 nucleotides long RNA is transcribed in vitro from a very strong promoter located close to the origin of replication of plasmid ColE1 (21). Since the his plasmids are pBR322 and pBR313 derivatives, the 100 nucleotides long RNA (Fig. 3) may be homologous to that found for ColE1 (20). In fact, a DNA sequence essentially identical to the strong promoter of ColE1 (21), to the 104 bases long ColE1 transcript (20), and to its transcription termination site (23) is present in pBR322 (24) at the homologous site close to the origin of replication.

Comparison of the relative rate of synthesis of the leader RNA and the 100 nucleotides long RNA indicates that the his promoter is a very strong promoter in vitro. This result is in keeping with the in vivo observation that, in mutants deleted of the attenuator, the amount of the first biosynthetic enzyme reaches about 3-4% of total cell proteins (25).

The efficiency of transcription of the his leader RNA is drastically decreased when the template is in its linear form (Fig. 3, Panel II). The same is true for the 100 nucleotides RNA as was already observed for ColE1 (20). This effect seems to be preferential for certain promoters, like ColE1 (20), rrnB (26) and λP_L (27). In this respect the his promoter seems to be among the most affected ones. This result indicates that the secondary structure of the DNA template may be of great relevance in determining promoter efficiency and perhaps promoter selection by RNA polymerase.

The nucleotide sequence of the HinfI 730 bp fragment has several interesting features. It contains two transcription initiation sites (nucleotides 260-222, for the "A" RNA; nucleotides 400-447, for the his leader RNA), a very strong transcription termination site for the his leader RNA (nucleotides 590-627), and an inter-promoters region (nucleotides 300-370). The features of the his leader RNA and of its termination site (attenuator) have already been discussed (1, 2).

The "A" promoter. Seven nucleotides before the start of the "A" RNA (nucleotide 222, 18), a possible Pribnow box is encountered (CATTGT). A very weak homology to the consensus sequence of the -35 recognition region is also found. The region between the Pribnow box and the -35 recognition sequence is very A-T rich, which is a rather common feature among promoters. We do not yet know the physiological function of this promoter.

The his leader promoter. Transcription of the his leader RNA starts at

nucleotide 447 (Fig. 5), both in vitro and in vivo (18). This defines the his promoter. A comparison of the his promoter sequence with that of the promoters of other biosynthetic operons (1) does not reveal any particular similarity. All sequences compared (his, trp, leu, thr, ilv) have, in addition to the Pribnow box, a -35 TTG sequence in line with the observation that this sequence is present in promoters that do not require proteins other than RNA polymerase to initiate transcription (22).

The inter-promoters region. The overall A-T content of the HinfI 730 bp fragment is reported in Fig. 6. The two promoters are extremely A-T rich and are separated by a G-C rich region. The overall A-T content of the transcribed regions approaches the average base composition of the E. coli chromosome. The A-T richness is a common feature of transcription initiation regions along the bacterial chromosome. It has also been described, for instance, for the promoter of the outer membrane lipoprotein gene of E. coli (28). A correlation between promoters and A-T content has been established (29).

The DNA between the "A" promoter and the his promoter has a very peculiar structure. A perfect 10 bp palyndrome (nucleotides 325-347) is found just in the middle of the G-C rich region (nucleotides 300-369) (Fig. 6).

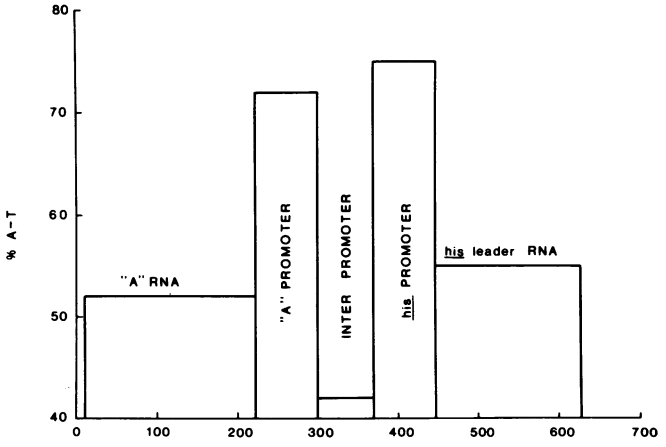

Fig. 6. Distribution of A-T base pairs in the E. coli his operon regulatory region. The region of DNA is divided in the following five sections. Nucleotides 10-222: "A" RNA transcribed region (52% A-T). Nucleotides 223-299: a very A-T rich region (72%) containing the "A" promoter. Nucleotides 300-369: the inter-promoters region G-C rich (42% A-T). Nucleotides 370-446: a very A-T rich region (75%) containing the his promoter. Nucleotides 447-627: his leader RNA transcribed region (55% A-T).

Such a structure might be regarded as a "divide" between two easily melting A-T rich regions where RNA polymerase is allowed to bind. It is conceivable to think, for instance, that these regions prevent interference between two RNA polymerase molecules initiating transcription at closely located promoters.

Ribosomal binding sites. The DNA region before the putative his leader peptide sequences (nucleotides 478-528, Fig. 5) presents only limited homology to the 3' sequence of the 16S ribosomal RNA (Shine-Dalgarno box, 30), nucleotides GAG at position 469-471. The overall sequence, however, is compatible with the ideal E. coli ribosomal binding site (31). On the contrary, the Shine-Dalgarno sequence preceding the beginning of the hisG gene is very well preserved. The homologous sequence is TAANGAGGNNNNNCA, between nucleotides 655 and 670. The amino acid sequence of the hisG protein of E. coli has not been determined. A comparison of the DNA sequence between nucleotides 672-717 and the derived amino acids, with the published amino acid sequence of the hisG enzyme of S. typhimurium (32) shows that 12 of the 15 amino acids are identical.

ACKNOWLEDGEMENTS

We would like to thank Drs. R. Cortese, R. Di Lauro, G. Macino and M. Rosenberg for helpful discussions and criticism during the course of this work.

ABBREVIATIONS[§]

bp, base pairs; EDTA, ethylenediamine tetracetic acid; SDS, sodium dodecyl sulfate; N, any nucleotide.

* Present address: International Institute of Genetics and Biophysics of the C.N.R., Via G. Marconi 10, Naples, Italy.

REFERENCES

1. Blasi,F. and Bruni,C.B. (1981) in Current Topics in Cellular Regulation, Horecker,B.L. and Stadtman,E.R. Eds., Vol.XIX, Academic Press, New York, in press.
2. Di Nocera,P.P., Blasi,F., Di Lauro,R., Frunzio,R. and Bruni,C.B. (1978) Proc. Natl. Acad. Sci. USA 75, 4276-4280.
3. Barnes,W.M. (1978) Proc. Natl. Acad. Sci. USA 75, 4281-4285.
4. Johnston,H.M., Barnes,W.M., Chumley,F.G., Bossi,L. and Roth,J.R. (1980) Proc. Natl. Acad. Sci. USA 77, 508-512.
5. Keller,E.B. and Calvo,J.M. (1979) Proc. Natl. Acad. Sci. USA 76, 6186-6190.

6. Bruni,C.B., Di Lauro,R., Di Nocera,P.P., Carlomagno,M.S., Frunzio,R., Musti,A.M. and Blasi,F. (1978) in Genetic Engineering, Boyer,H.W. and Nicosia,S. Eds., pp. 143-150, Elsevier-North Holland, Amsterdam.
7. Bruni,C.B., Musti,A.M., Frunzio,R. and Blasi,F. (1980) J. Bacteriol. 142, 32-42.
8. Bickle,T.A., Pirrotta,V. and Imber,R. (1977) Nucleic Acids Res. 4, 2561-2572.
9. Glynn,I.M. and Chappell,J.B. (1964) Biochem. J. 90, 147-153.
10. Jones,B.B. and Reznikoff,W.S. (1977) J. Bacteriol. 132, 270-281.
11. Thomas, Jr.,C.A., Berns,K.I. and Kelly,Jr.,T.J. (1966) in Procedures in Nucleic Acids Research, Cantoni,G.L. and Davies,D.R. Eds., pp. 535-537, Harper and Row, New York.
12. Clewell,D.B. (1972) J. Bacteriol. 110, 667-676.
13. Maxam,A.M. and Gilbert,W. (1979) in Methods in Enzymology, Grossman,L. and Moldave,K. Eds., Vol. LXV, pp. 499-560, Academic Press, New York.
14. Bolivar,F., Rodriguez,R.L., Betlach,M.C. and Boyer,H.W. (1977) Gene 2, 75-93.
15. Inoko,H., Shigescida,K. and Imai,M. (1977) Proc. Natl. Acad. Sci. USA 74, 1162-1166.
16. Goldschmidt,E.P., Cater,M.S., Matney,T.S., Butler,W.A. and Greene,A. (1970) Genetics 66, 219-229.
17. Sanger,F. and Coulson,A.R. (1978) FEBS Letters 87, 107-116.
18. Frunzio,R., Bruni,C.B. and Blasi,F. (1981) Proc. Natl. Acad. Sci. USA, in press.
19. Borck,K., Beggs,S.J.D., Brammar,W.J., Hopkins,A.S. and Murray,N.E. (1976) Mol. Gen. Genet. 146, 199-207.
20. Levine,A.D. and Rupp,W.D. (1978) in Microbiology, Schlessinger,D. Ed., pp. 163-166, Amer. Soc. Microbiology.
21. Chan,P.T., Lebowitz,J. and Bastia, D. (1979) Nucleic Acids Res. 7, 1247-1262.
22. Rosenberg,M. and Court,D. (1979) Ann. Rev. Genet. 13, 319-353.
23. Oka,A., Nomura,N., Morita,M., Sugisaki,H., Sugimoto,K. and Takanami,M. (1979) Molec. Gen. Genet. 172, 151-159.
24. Sutcliffe,J.G. (1978) Cold Spring Harbor Symp. Quant. Biol. 43, 77-90.
25. Parsons,S.M. and Koshland,D.E.Jr. (1974) J. Biol. Chem. 249, 4104-4109.
26. Yang,H.L., Heller,K., Gellert,M. and Zubay,G. (1979) Proc. Natl. Acad. Sci. USA 76, 3304-3308.
27. Smith,C., Kubo,M. and Imamoto,F. (1978) Nature (London) 275, 420-423.
28. Nakamura,K. and Inouye,M. (1979) Cell 18, 1109-1117.
29. Vollenweider,H.J., Fiandt,M. and Szybalski,W. (1979) Science 205, 508-511.
30. Steitz,J.A. (1978) in Biological Regulation and Control, Goldberger,R.F. Ed., Vol. I, pp. 349-399, Plenum Publishing Co.
31. Scherer,G.F.E., Walkinshaw,M.D., Arnott,S. and Morré,D.J. (1980) Nucleic Acids Res. 8, 3895-3907.
32. Piskiewicz,D., Tilley,B.E., Rand-Meir,T. and Parsons,S.M. (1979) Proc. Natl. Acad. Sci. USA 76, 1589-1592.