JOURNAL OF BACTERIOLOGY, Sept. 1984, p. 934–939
0021-9193/84/090934-06$02.00/0

Vol. 159, No. 3

Regulation of *hutUH* Operon Expression by the Catabolite Gene Activator Protein-Cyclic AMP Complex in *Klebsiella aerogenes*

ANTHONY J. NIEUWKOOP, SHARON A. BOYLAN, AND ROBERT A. BENDER*

Department of Cellular and Molecular Biology, Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan 48109

Received 14 May 1984/Accepted 11 June 1984

RNA polymerase transcribed the *hutUH* operon of *Klebsiella aerogenes* if the catabolite gene activator protein (CAP) and cyclic AMP (cAMP) were present or if the DNA template was derived from a promoter mutant in which *hutUH* expression was independent of the need for positive effectors. In the absence of CAP or cAMP, not only was *hutUH* transcription absent, but transcription in the opposite direction (toward *hutC*) was initiated at a site (p_C) ca. 70 base pairs from the site (p_{UH}) of *hutUH* mRNA initiation. When the p_C promoter was cloned in front of a promoterless *galK* gene, active expression of *galK* was observed. Thus, the p_C promoter is active in vivo as well as in vitro. Transcription from p_{UH} and p_C may be mutually exclusive, with the major effect of CAP and cAMP being to prevent transcription from p_C, thus relieving the antagonistic effect on transcription from p_{UH}. This "double-negative" control by CAP-cAMP is supported by two observations: (i) CAP-cAMP was unable to activate transcription from p_{UH} if RNA polymerase had been previously bound to p_C and (ii) a mutation that allowed transcription from p_{UH} in the absence of positive effectors simultaneously eliminated the activity of p_C. An alternative model, in which CAP-cAMP is required for p_{UH} expression and RNA polymerase binding at p_C serves to modulate this control in some unknown way, is also considered. The physiological role of the transcript from p_C other than regulation of p_{UH} is unknown.

Klebsiella aerogenes can degrade histidine to glutamate, ammonia, and formamide by a sequence of four enzymatic reactions (9). The genes for the histidine-utilization (*hut*) operons are located between *gal* and *bio* on the *K. aerogenes* chromosome and are arranged in the order *hut(M)IGC(P)UH* (2, 7). The *hutI, G, U,* and *H* genes encode the four enzymes of the pathway; *hutC* encodes a repressor whose effect is neutralized by urocanate, the product of the first enzyme in the pathway (15). The *hut* genes are arranged in at least two operons in which transcription of *hutI* and *hutU* are independently initiated (2). We have modified the nomenclature established for the *hut* operons of *Salmonella typhimurium* (4) in that we use *hut(M)* and *hut(P)* to describe the control regions that contain the promoters of the *hutI* and *hutU* transcription units, respectively (2). By this convention, the *hut(P)* region, the focus of the work reported here, is expected to encode the promoter, operator, and positive-effector-binding sites of the *hutUH* operon.

Histidine can serve as the sole carbon or nitrogen source for *K. aerogenes*, and high-level expression of the *hut* operons requires the presence of a positive effector in addition to repressor inactivation. This effector can be either the catabolite gene activator protein (CAP) in the presence of cyclic AMP (cAMP) (14), signaling carbon and energy limitation, or an unknown factor signaling nitrogen limitation (14). CAP-cAMP was defined as a positive effector because mutants lacking either CAP or adenylate cyclase activity were unable to produce high levels of *hut* products except when starved for ammonia (14). From genetic and physiological studies (9, 14) and a preliminary analysis of in vitro transcription of the *hut* genes of *S. typhimurium* (17), CAP-cAMP regulation of the *hut* operons appeared analogous to CAP-cAMP regulation of the *Escherichia coli lac* operon. In fact, when the *K. aerogenes hut* genes are transferred into *E. coli*, which lacks *hut* genes, the regulation of histidase formation by carbon limitation parallels that of β-galactosidase formation (6, 13).

The molecular basis for the positive regulation of *hut* transcription is unknown (14). We present data that the positive regulation responding to carbon and energy limitation is actually a "double-negative" control. In this system, RNA polymerase ordinarily binds to a newly identified site near the *hutUH* promoter, and RNA polymerase bound at this site blocks the binding of RNA polymerase to the *hutUH* promoter. CAP-cAMP prevents the binding of RNA polymerase to the "unproductive" site, which in turn allows free access of RNA polymerase to the "productive" *hutUH* promoter.

MATERIALS AND METHODS

Enzymes and templates. RNA polymerase and CAP were purified in this laboratory from *K. aerogenes*, essentially by the method of Lowe et al. (8). Details will be described elsewhere. Template DNA was a 3.1-kilobase (kb) *Bgl*II fragment prepared from plasmid pCB101 (3), which carries the wild-type *hut* operon, or from plasmid pCB209 (manuscript in preparation), which carries the *hutP104* mutation rendering *hutUH* expression independent of positive effectors. The 3.1-kb *Bgl*II fragment, which contains no promoters outside the *hut(P)* regions, was purified from the larger fragment by sucrose density gradient centrifugation in the presence of ethidium bromide as previously described (3). Template DNA was cleaved with a variety of restriction enzymes according to the directions of the supplier.

The transcriptional assay was basically that described by Maquat and Reznikoff (10). Reaction mixtures (25 μl) contained 30 mM Tris-hydrochloride (pH 7.9), 0.1 mM EDTA, 10 mM $MgCl_2$, 100 mM KCl, 0.1 mM dithiothreitol, and 27 μg of bovine serum albumin per ml. The template DNA (0.3 μg) was preincubated for 5 min at room temperature either with or without CAP (1.1 μg) and cAMP (1 mM). RNA polymerase (0.48 μg) was then added, and the mixture was

* Corresponding author.

incubated for 30 min at 30°C. Heparin (2.5 μg) was added, and 45 s later the reaction was made 100 μM in ATP and GTP, 5 μM in CTP, and 1 to 5 μM in UTP, with [α-^{32}P]UTP at a specific activity greater than 100 Ci/mmol (New England Nuclear Corp.). Synthesis was terminated after 10 min of incubation at 37°C by the addition of 50 μl of stop mix (100 mM sodium acetate [pH 5.5], 0.4% sodium dodecyl sulfate, and 1 mg of torula yeast RNA, type VI [Sigma Chemical Co.]). The mixture was extracted with 75 μl of phenol-sevag, followed by extraction with chloroform. Samples were then precipitated with 250 μl of 95% ethanol containing 100 mM NaAc (pH 6.0) and 10 mM $MgCl_2$ at −70°C for 20 min. The precipitate was collected by centrifugation, dried in a vacuum for ca. 30 min, and suspended in 6 μl of 0.1 M Tris-borate containing 0.2% bromophenol blue and 80% deionized formamide. Samples were boiled for 5 min and then placed on ice. The transcripts were separated by electrophoresis in polyacrylamide gels containing 7 M urea and TBE (0.1 M Tris-borate [pH 8.3], 1 mM EDTA) gel. An autoradiograph was made of the gels by exposing them to X-ray film (Kodak XAR-5) at −70°C.

RESULTS

Identification of mRNA transcript from *hutUH*. In vivo, expression of the wild-type *hutUH* operon requires activation by a positive effector, either CAP-cAMP or an unidentified nitrogen starvation factor (14). The *hutP104* mutation obviates the requirement for a positive effector, allowing high-level expression of the *hutUH* operon in vivo, even in the presence of excess carbon and nitrogen sources (manuscript in preparation). By using *hut* DNA carrying the *hutP104* mutation, we were able to identify the *hutUH* mRNA produced in an in vitro transcription reaction by RNA polymerase alone. A *Bgl*II restriction fragment, which contains *hut* DNA from the middle of the *hutG* gene to the middle of the *hutH* gene inclusive (2), was purified as described above and further digested with *Hae*III, *Kpn*I, or *Pvu*II, in each case yielding a collection of fragments. Each of these collections of restriction fragments was incubated with RNA polymerase purified from *K. aerogenes* (manuscript in preparation), and in each instance a single major runoff transcript was detected, consistent with the presence of a single strong promoter on the *Bgl*II fragment of *hutP104* DNA (Fig. 1). Since the restriction map of this region is known (3), the length of the transcripts could be compared with the location of the restriction cleavage sites that define the 3′ end of these runoff transcripts. This comparison demonstrated a single point of origin for the transcripts (position 3.53 on the standard map) and a single direction of transcription (rightward) (Fig. 1). This start point corresponds to the region known to contain *hut(P)* (2), and this direction would carry the transcript into the *hutUH* operon from 5′ to 3′. Thus, we have identified an in vitro RNA that is most likely the *hutUH* transcript.

Regulation of *hutUH* transcription by CAP-cAMP. To confirm that we had detected the *hutUH* transcript, it was necessary to demonstrate that RNA polymerase would produce this transcript from wild-type *hut* fragments if and only if a positive effector was present. The *hutUH* runoff transcript was produced from wild-type (*hutP*$^+$) DNA if cAMP

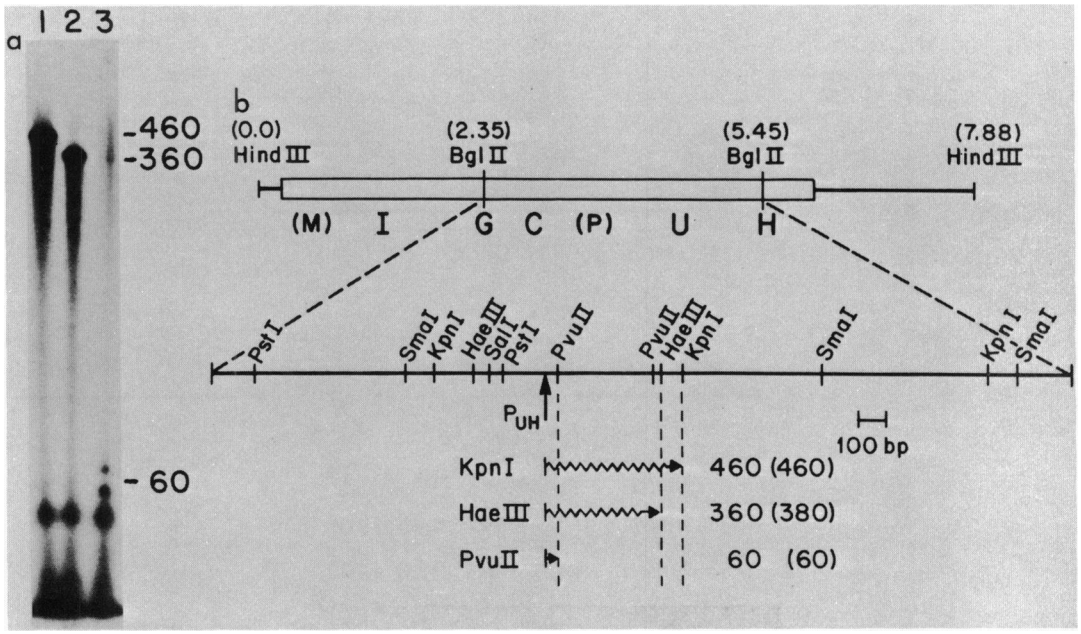

FIG. 1. Identification of a rightward transcript from *hut(P)*. (a) Template DNA (carrying the *hutP104* mutation) was purified as described in the text. The template was then digested with *Kpn*I, *Hae*III, and *Pvu*II restriction enzymes, yielding 4, >20, and 3 fragments, respectively (3). The radioactive products of runoff transcription were separated electrophoretically in a 10% polyacrylamide gel and visualized by autoradiography. Lane 1, *Kpn*I-digested DNA as template; lane 2, *Hae*III-digested DNA as template; lane 3, *Pvu*III-digested DNA as template. Sizes were determined with end-labeled fragments resulting from digestion of φX174 RFII with *Hae*III and pBR322 with *Hin*fI. (b) Alignment of runoff transcript lengths with the restriction map of the *hut(P)* region. The transcripts, represented by wavy lines, are aligned such that the 3′ end lies at a restriction site for the enzyme used to cleave the template. Only one combination results in a unique start site (as shown). The numbers beside the wavy lines indicate the size of the transcripts (in bases) estimated from the mobility in denaturing gels; the numbers in parentheses indicate the size calculated from the restriction map (3), assuming that transcription is rightward and initiates at map position 3.53, where map position is measured in kb pairs, with 0.0 defined as the *Hin*dIII site at the left as shown.

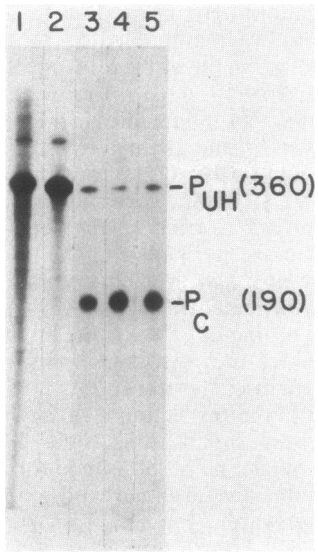

FIG. 2. Runoff transcripts generated from wild-type *hut(P)* region DNA in the presence and absence of CAP and cAMP. The transcription reactions were as in Fig. 1, using template DNA purified from a wild-type *hut* operon (in plasmid pCB101). The template was cleaved with *Hae*III (cf. with Fig. 1, lane 2), and runoff transcripts were produced. Treatments other than the standard reaction were: template DNA preincubated with CAP and cAMP before the addition of RNA polymerase (lane 2), preincubation with CAP but no cAMP (lane 3), preincubation with cAMP but no CAP (lane 4), and no preincubation (lane 5). Lane 1 is a control in which *hutP104* template DNA was used (as in Fig. 1, lane 2). Sizes were determined as described in the legend to Fig. 1.

and CAP were present at the time when RNA polymerase was added, but this transcript was virtually absent if either CAP or cAMP was omitted from the preincubation mixture (Fig. 2). Thus, RNA polymerase transcribes *hutUH* mRNA from a wild-type template only in the presence of CAP and cAMP. An identical result was obtained if RNA polymerase from *E. coli* replaced the *K. aerogenes* protein (data not shown).

When CAP, cAMP, or both were omitted from the wild-type reaction mixtures not only was the *hutUH* transcript lost, but a completely different runoff transcript appeared. This transcript was not seen with either *hutP104* DNA or CAP-cAMP-treated *hutP*$^+$ DNA. The origin of this transcript was at position 3.46, and transcription proceeded leftward, opposite to that of the *hutUH* transcript (Fig. 3). Thus, within the *hut(P)* region of *K. aerogenes* there is a bidirectional promoter. Since the rightward transcript appears to read the *hutUH* operon and is stimulated by CAP-cAMP, we refer to the promoter oriented in this direction as p_{UH}. The leftward transcript proceeds toward the *hutC* gene, and we designate its promoter as p_C.

In vivo activity of the p_C promoter. An RNA transcript reading leftward from *hut(P)* toward *hutC* had not previously been identified. We therefore determined whether the postulated p_C promoter was functional in vivo. A 1.5-kb-pair fragment generated by digestion of wild-type *hut* DNA of plasmid pCB101 (3) with *Ava*I was cloned into plasmid pK01 in front of the promoterless *galK* gene (12). This *Ava*I fragment is identical to the 1.5-kb *Sma*I fragment from map coordinates 3.05 to 4.55 (Fig. 1 and 3) and contains the entire *hut(P)* region (2). The orientation of the 1.5-kb *hut(P)* fragment within this plasmid, pCB202, was determined by

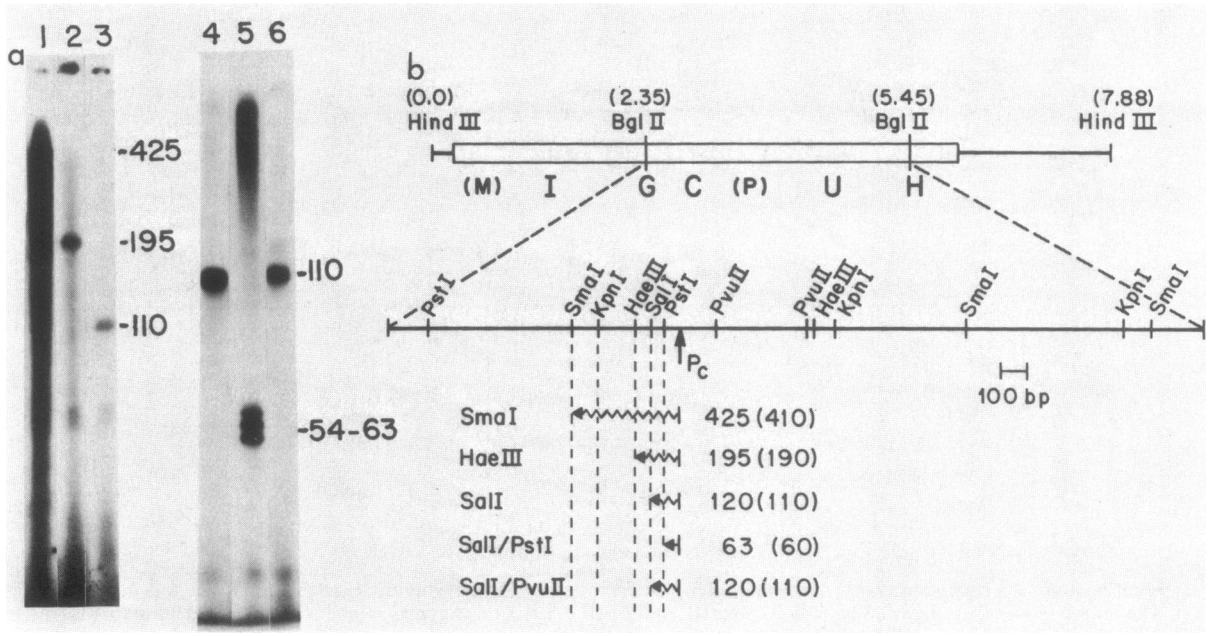

FIG. 3. (a) Identification of a leftward transcript from *hutP*. Reaction conditions were as described in the legend to Fig. 1, except that wild-type (*hutP*$^+$) template DNA was used in all reactions. The template DNA was cleaved with *Sma*I (lane 1), *Hae*III (lane 2), or *Sal*I (lane 3), and transcripts were electrophoretically separated in a 7% polyacrylamide gel. In the remaining experiments, the template DNA was cleaved either with *Sal*I (lane 4) or *Sal*I and subsequently with *Pst*I (lane 5) or *Pvu*II (lane 6). The transcripts in lanes 4 to 6 were separated in a 10% polyacrylamide gel. Sizes were estimated as described in the legend to Fig. 1. (b) Alignment of runoff transcript lengths with the restriction map of the *hut(P)* region. Symbols are as described for Fig. 1b, except that the numbers in parentheses indicate the size predicted from the restriction map (3) assuming that transcription is leftward and initiates at map position 3.46.

analysis of the fragments produced by digestion of pCB202 with enzymes that cleave the fragment asymmetrically (data not shown). This analysis showed that the inserted fragment was oriented such that the promoterless *galK* gene would be under the control of p_C. When either the original pK01 plasmid or no plasmid was present in strain N100 (*galK recA*), the strain gave a negative response for galactose fermentation on a MacConkey indicator plate. However, when pCB202 was present in strain N100 (after transformation), the MacConkey plate scored strongly positive for galactose fermentation. Thus, the plasmid-borne *galK* gene was expressed from the putative p_C promoter, demonstrating that p_C is active in vivo as well as in vitro.

Negative regulation by CAP-cAMP. The p_C transcript was not produced by wild-type DNA if the DNA had been preincubated with CAP-cAMP, suggesting negative regulation of the p_C transcript by CAP-cAMP. To confirm this suggestion, we varied the order of addition of the RNA polymerase and the CAP-cAMP. When CAP-cAMP was added before RNA polymerase, little or no p_C transcript was detected (Fig. 4). However, when RNA polymerase was added before the CAP-cAMP complex, even a further 30-min incubation in the presence of CAP-cAMP failed to prevent p_C transcription. Thus, RNA polymerase and CAP-cAMP appear to compete for binding at the p_C promoter.

Interaction between the p_{UH} and P_C promoters. When CAP-cAMP was present in the reaction mixture, it simultaneously blocked p_C expression and allowed or stimulated p_{UH} expression. Moreover, once RNA polymerase had been allowed to form an open complex with *hutP*$^+$ DNA at the p_C promoter, CAP-cAMP was unable to allow or stimulate p_{UH} expression even after prolonged incubation (Fig. 4). Therefore, we hypothesized that binding of RNA polymerase at p_C precludes binding of RNA polymerase at p_{UH}. This model is further supported by the fact that the single mutational event that generated the *hutP104* mutation, allowing *hutUH* expression without the need to bind CAP-cAMP, simultaneously eliminated p_C promoter activity completely in vitro (Fig. 1). One alternative explanation of the *hutP104* phenotype is that *hutP104* created a "super p_{UH} promoter" that captures RNA polymerase, leaving no RNA polymerase available to transcribe from p_C. If this super promoter model were correct, then the super promoter should be able to capture RNA polymerase molecules from the p_C promoter, whether the p_C promoter were on the same piece of DNA or on a separate piece. Both *hutP*$^+$ and *hutP104* templates, cut with different enzymes to allow resolution of all four possible transcripts, were added to the same reaction mixture. In this mixing experiment, the *hutP104* template produced only the p_{UH} transcript, and the *hutP*$^+$ template produced the p_C transcript. The presence of the *hutP104* DNA did not alter the ability of p_C to be expressed except from the mutant fragment, confirming that the interference between RNAp_C binding and RNAp_{UH} binding is steric rather than competitive.

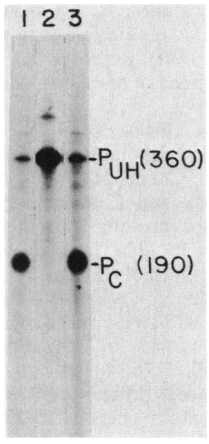

FIG. 4. Mutual antagonism of RNa polymerase and CAP binding. Template was wild-type (*hutP*$^+$) DNA cleaved with *Hae*III (cf. with Fig. 2). Lane 1, CAP and cAMP were omitted; lane 2, template was preincubated with CAP and cAMP for 10 min before the addition of RNA polymerase; lane 3, template was preincubated with RNA polymerase for 10 min before the addition of CAP and cAMP. Incubation was continued for an additional 30 min before the addition of nucleotides to start transcription.

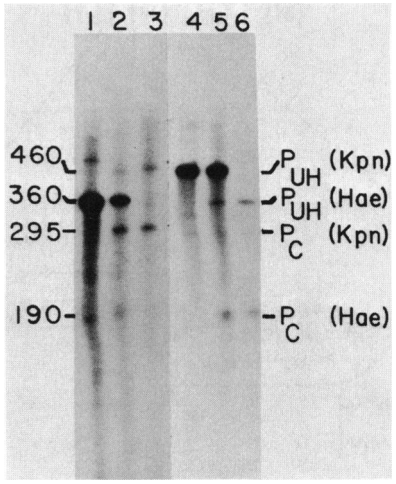

FIG. 5. *Cis*-dominance of the *hutP104* mutation in vitro. Reaction conditions were as described in the legend to Fig. 1. In the first experiment, wild-type (*hutP*$^+$) DNA was cleaved with *Kpn*I and mutant (*hutP104*) DNA with *Hae*III. Thus, the p_{UH} and p_C transcripts from *hutP*$^+$ are 460 and 295 bases long; those from *hutP104* are 360 and 190 bases long (Fig. 1 and 3). Lane 1, the reaction mixture contained *hutP104* template cleaved with *Hae*III; lane 3, *hutP*$^+$ template cleaved with *Kpn*I; lane 2, a mixture of the DNAs in lanes 1 and 3. The second experiment was reciprocal in that the restriction enzymes were reversed. Lane 4, the reaction mixture contained *hutP104* template cleaved with *Kpn*I; lane 6, *hutP*$^+$ template cleaved with *Hae*III; lane 5, a mixture of the DNAs in lanes 4 and 6.

DISCUSSION

The data presented here support a novel role for the CAP-cAMP complex in the catabolite repression control of the *hutUH* operon of *K. aerogenes*. This model of double-negative control by CAP-cAMP is shown in Fig. 6. In the absence of CAP or cAMP, RNA polymerase preferentially chooses a promoter in the *hut(P)* region directed away from the *hutUH* operon. The binding of RNA polymerase to this p_C promoter precludes the binding of RNA polymerase to the promoter of the *hutUH* operon (p_{UH}), presumably by steric hindrance. If RNA polymerase binding to p_C can be prevented by pretreating the DNA template with CAP-cAMP, then RNA polymerase recognizes the p_{UH} promoter and transcribes into the *hutUH* operon.

This model can be separated into two components: that CAP-cAMP competes with RNA polymerase for binding at the p_C site and that binding of RNA polymerase at the p_C and

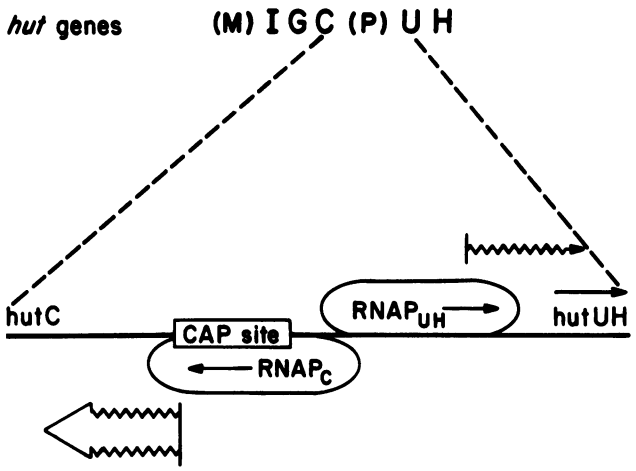

FIG. 6. Model for CAP-cAMP regulation of transcription from *hut(P)*.

p_{UH} sites is mutually exclusive. The first part of the model, competition at p_C, is strongly documented by the data in Fig. 4, showing that CAP-cAMP is unable to affect *hut* transcription if RNA polymerase has formed an open complex at p_C. The second part of the model, direct negative control by RNA polymerase bound at p_C, is the most likely explanation for the observations that (i) CAP-cAMP affects both p_C and p_{UH} but in opposite directions, (ii) the *hutP104* mutation simultaneously destroys the p_C promoter activity and makes p_{UH} activity independent of the CAP-cAMP requirement, and (iii) the *hutP104* mutation does not alter the expression of *hutP*$^+$ DNA in a mixing experiment. It remains possible, however, that the negative control of transcription from p_{UH} by RNA polymerase bound at p_C is indirect, with p_C-bound RNA polymerase blocking the binding of CAP-cAMP which would be absolutely required for p_{UH} activity. This model would be consistent with the three observations listed above only if we assume that the *hutP104* mutation simultaneously strengthened the p_{UH} promoter and destroyed the p_C promoter. Since the distance between mRNA start sites is ca. 70 base pairs, p_C and p_{UH} clearly overlap, so such a double phenotype is not impossible. This alternative model, however, fails to provide a physiological role for the competition between CAP-cAMP and RNA polymerase at p_C, hence our preference for the double-negative control model. DNA sequence analysis currently in progress should help distinguish between the two models. In either case, the data presented here argue that RNA polymerase bound at p_C negatively regulates transcription from p_{UH} either directly or by blocking the binding of CAP-cAMP.

The concept of CAP-cAMP exerting its positive effect on transcription by blocking the binding of RNA polymerase to an "unproductive promoter" is not unique either to *K. aerogenes* or to the *hut* operons. In the *gal* operon (5), transcription from p_2 (a cAMP-independent promoter) prevents transcription from p_1 located 5 base pairs downstream from p_2. The CAP-cAMP complex in turn represses transcription from p_2 and thus relieves the block on p_1. It has not yet been shown whether RNA polymerase bound to p_2 can block the action of the CAP-cAMP complex. The *gal* model is analogous to the *hut* model proposed here, except that in *gal* both p_2 and p_1 are oriented in the same direction, whereas in *hut* p_C and p_{UH} are oriented in opposite directions.

McClure and his colleagues have proposed an analogous mechanism for CAP-cAMP regulation of the *lac* operon of *E. coli* (11). In their model, CAP-cAMP blocks binding of RNA polymerase to a promoter from which transcription only rarely initiates. Prebinding of CAP-cAMP prevents this ineffective binding and aids binding of RNA polymerase to the well-known *lac* promoter in which transcription initiation is rapid. The *hut* model differs from the *lac* model in three respects: (i) the second *hut(P)* region promoter, p_C, is oriented opposite to the main promoter, p_{UH}, whereas both promoters in *lac* are oriented toward *lacZ*; (ii) the p_C promoter is functional in vivo, whereas no in vivo activity has yet been demonstrated for the second *lac* promoter; (iii) the choice between p_C and p_{UH} is essentially dynamic since RNA polymerase vacates both p_C and p_{UH} by transcribing, whereas the choice in *lac* is basically static since RNA polymerase bound at the ineffective site is postulated to remain there for a long time before leaving.

The role of the p_C transcript is unknown. Although transcription proceeds toward a region known to contain the *hutC* gene (2), the evidence both from *S. typhimurium* and from *K. aerogenes* (1, 16; unpublished data) strongly suggests that *hutC* is transcribed from left to right. The data presented here demonstrate a regulatory role for transcription from p_C vis a vis transcription from p_{UH}, and the p_C transcript may in fact not encode any protein.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AI15822 and GM27111 from the National Institutes of Health. S.A.B. was supported by postdoctoral fellowship PF1739 from the American Cancer Society and by a grant from the Biomedical Research Council of the University of Michigan.

LITERATURE CITED

1. **Blumenberg, M., and B. Magasanik.** 1979. A study in evolution: the histidine utilization genes of enteric bacteria. J. Mol. Biol. **135**:23–27.
2. **Boylan, S. A., and R. A. Bender.** 1984. Genetics and physical maps of *Klebsiella aerogenes* genes for histidine utilization (*hut*). Mol. Gen. Genet. **193**:99–103.
3. **Boylan, S. A., L. J. Eades, K. A. Janssen, M. I. Lomax, and R. A. Bender.** 1984. A restriction enzyme cleavage map of the histidine-utilization (*hut*) genes of *Klebsiella aerogenes* and deletions lacking regions of *hut* DNA. Mol. Gen. Genet. **193**:92–98.
4. **Brill, W. J., and B. Magasanik.** 1969. Genetic and metabolic control of histidase and urocanase in *Salmonella typhimurium*, strain 15–59. J. Biol. Chem. **244**:5392–5402.
5. **de Crombrugghe, B., and I. Pastan.** 1978. Cyclic AMP, the cyclic AMP receptor protein, and their dual control of the galactase operon, p. 303–324. *In* J. H. Miller and W. S. Reznikoff (ed.), The operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Goldberg, R. B., F. R. Bloom, and B. Magasanik.** 1976. Regulation of histidase synthesis in intergeneric hybrids of enteric bacteria. J. Bacteriol. **127**:114–119.
7. **Goldberg, R. B., and B. Magasanik.** 1975. Gene order of the histidine utilization (*hut*) operons in *Klebsiella aerogenes*. J. Bacteriol. **122**:1025–1031.
8. **Lowe, P. A., D. A. Hager, and R. R. Burgess.** 1979. Purification and properties of the σ subunit of *Escherichia coli* DNA-dependent RNA polymerase. Biochemistry **18**:1344–1352.
9. **Magasanik, B.** 1978. Regulation in the *hut* system, p. 373–387. *In* J. H. Miller and W. S. Reznikoff (ed.), The operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **Maquat, L. E., and W. S. Reznikoff.** 1978. In vitro analysis of the *Escherichia coli* RNA polymerase interactions with wild-

type and mutant lactose promoters. J. Mol. Biol. **125**:467–490.
11. **McClure, W. R., D. K. Hawley, and T. P. Malan.** 1982. The mechanism of RNA polymerase activation on the λ P_{RM} and *lacP*$^+$ promoters. *In* R. L. Rodriguez and M. J. Chamberlin (ed.), Promoters: structure and function. Praeger Scientific Publishing Co.
12. **McKenney, K., H. Shimatake, D. Court, U. Schmeissner, C. Brady, and M. Rosenberg.** 1981. A system to study promoter and terminator signals recognized by *Escherichia coli* RNA polymerase, p. 383–415. *In* J. S. Chirikjian and T. S. Papas (ed.), Gene amplification and analysis. Elsevier/North-Holland Publishing Co., New York.
13. **Parada, J. L., and B. Magasanik.** 1975. Expression of the *hut* operons of *Salmonella typhimurium* in *Klebsiella aerogenes* and in *Escherichia coli*. J. Bacteriol. **124**:1263–1268.
14. **Prival, M. J., and B. Magasanik.** 1971. Resistance to catabolite repression of histidase and proline oxidase during nitrogen-limited growth of *Klebsiella aerogenes*. J. Biol. Chem. **246**:6288–6296.
15. **Schlesinger, S., P. Scotto, and B. Magasanik.** 1965. Exogenous and endogenous induction of the histidine-degrading enzymes in *Aerobacter aerogenes*. J. Biol. Chem. **240**:4331–4337.
16. **Smith, G. R., and B. Magasanik.** 1971. Nature and self regulated synthesis of the repressor of the *hut* operon in *Salmonella typhimurium*. Proc. Natl. Acad. Sci. U.S.A. **68**:1493–1497.
17. **Tyler, B., A. B. De Leo, and B. Magasanik.** 1974. Activation of transcription of *hut* DNA by glutamine synthetase. Proc. Natl. Acad. Sci. U.S.A. **71**:225–229.