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Nature and Self-Regulated Synthesis of the Repressor of the *hut* Operons in *Salmonella typhimurium*

(histidine utilization/phage λ/transduction)

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ABSTRACT The enzymes mediating histidine utilization, coded by the *hut* genes, in *Salmonella typhimurium* are synthesized from two closely-linked operons. Results presented in this paper show that these operons are regulated by a single repressor protein. The *hutC* gene, coding for this repressor, is part of one of the operons. Since this same operon is sensitive to the repressor, the repressor apparently represses its own synthesis.

The expression of certain bacterial and bacteriophage genes is controlled by repressor proteins synthesized by regulatory genes. Only when the repressor protein is inactivated are the genes expressed. The regulation of the regulatory genes themselves is an interesting problem. In the case of the *lac* operon of *Escherichia coli*, the repressor gene, *lacI*, appears to be expressed at the same level whether or not the operon is induced (1, 2). On the other hand, the protein made by one of the *pho* regulatory genes, *R2a*, is made in increased amounts under those genetic and environmental conditions which allow increased synthesis of alkaline phosphatase (3). In the case of phage λ, the repressor gene, *cI*, is controlled by a second gene, *tof*. The *tof* gene product prevents repressor synthesis during the lytic cycle. But during the lysogenic cycle the reverse is true: the repressor prevents expression of the *tof* gene (4–6). In the system we are studying, we have discovered that the regulatory gene makes a repressor which represses its own synthesis, in addition to repressing the synthesis of the enzymes of the system. We report here results leading to this conclusion and describe some properties of this regulatory mechanism.

We have examined the nature and regulation of the regulatory gene product in the *h*istidine *ut*ilization (i.e., *hut*) system of *Salmonella typhimurium*. The four enzymes of this system catalyze the conversion of histidine to glutamic acid, ammonia, and formamide. A detailed genetic analysis of this system was made possible by the isolation of F′ episomes (7) and λ transducing phages (8) carrying the *hut* genes.

The cluster of *hut* genes is located between the *gal* and *bio* loci on the chromosome (9–11). As shown in Fig. 1, these genes are arranged in two closely-linked operons. The right-hand operon consists of the structural gene (*H* and *U*) for the first two enzymes of the pathway and also the promoter–operator region (*P*, *R*, *Q*) regulating their expression. The *P*, *R*, and *Q* mutations act only in *cis* position on the *UH* operon (7). The left-hand operon contains the structural genes (*I* and *G*) for the last two enzymes of the pathway and a promoter (*M*), which regulates in *cis* position their level of expression (7, 11). The mutations in the *M*-promoter result in increased levels of expression of the *I* and *G* genes. Mutations, designated C^-‡, in the regulatory gene *hutC*, result in constitutive expression of both operons (9–11). In C^+/C^- heterozygous merodiploids, the wild-type C^+ allele is dominant to the mutant C^- allele, which suggests that the C^+ gene codes for a repressor (7).

MATERIALS AND METHODS

Bacterial strains

Derivatives of *S. typhimurium* strain 15-59 (prefix NE) and *E. coli* strain K-12 (prefix GS, MS, or MR) have been described previously (7–11), except for those described in Tables 1–5 and the following two strains, which were obtained from E. Signer: MS6041 (*lacZ NG1140*, *galK UV16*, Str^R, Su^-) and MS6040 (*lacZ NG1140*, *galK UV16*, Str^R, *suIII*$^+$). The *lac*$^-$ mutation in these strains is amber, and the strains are isogenic except for the presence of the amber supressor *suIII*$^+$ in MS6040.

Phage strains

The isolation of plaque-forming, specialized transducing phages, λ*phut*, has been described (8). The λ*phut* phages used here carry the *cI857* thermoinducible repressor allele and the *Ssus7* lysis-defective allele, except for those used in the experiment summarized in Table 2, which are S^+. The *hut* genes present on these phages are wild-type, except for *hutQ222* (see ref. 7) on λ*phut36* in strain GS139 (Table 2) and for *hutHl* (see ref. 10) on λ*phut36* in the strains described in Table 5.

Methods

Microbiological culture and genetic procedures have been described (7–11). Strains lysogenic for λ*cI857* derivatives were grown at 32°C. For the determination of enzyme activities, cells were grown in a minimal medium containing succinate as principal carbon source and ammonia as principal nitrogen source (11). For use with *E. coli* strains, the medium was supplemented with 1.0 g of trisodium citrate monohydrate and 1.0 mg of thiamine hydrochloride per liter. Cell-free ex-

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‡ In this paper, C^- is used to designate alleles, such as *C7*, in the *C* gene which result in constitutive synthesis of the Hut enzymes.

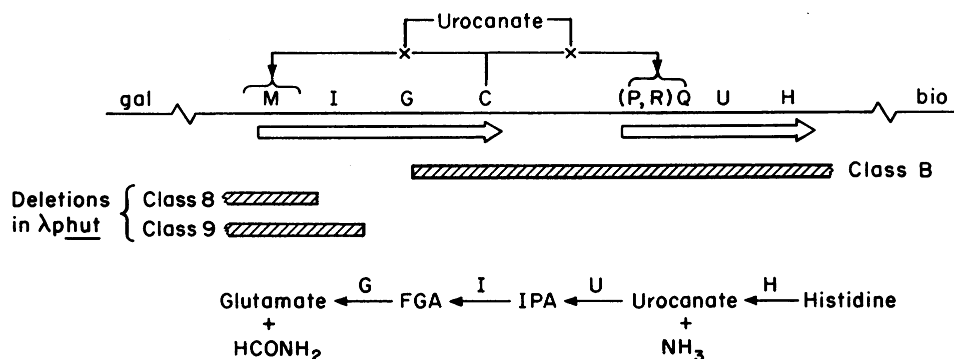

FIG. 1. Structure of the *hut* genetic region and pathway of histidine degradation. The capital letters indicate the *hut* genes and their products, which catalyze the indiacted reactions: H, L-histidine ammonia-lyase (histidase) (EC 4.3.1.3); U, urocanase; I, 4-imidazolone-5-propionate amidohydrolase (EC 3.5.2.7); G, *N*-formimino-L-glutamate formiminohydrolase. *M* is the promoter of the left-hand operon, *MIGC; P,R,Q* is the promoter-operator complex of the right-hand operon, *(P,R)QUH*. *C* is the repressor gene, whose presumed sites of action are indicated by the brackets. Its inactivation by urocanate is represented by crosses. The open arrows indicate the direction and extent of reading of the two operons. The hatched bars indicate the extents of the deletions used in Tables 2 and 5. Class B deletions remove the *G102*$^+$ allele and all *hut* genes to the right of it, but leave the *I105*$^+$ allele and *M* intact. Class 8 deletions leave *I105*$^+$ and all *hut* genes to the right of it intact but presumably remove *M*. Class 9 deletions leave *G102*$^+$ intact, but remove *I105*$^+$, and, presumably, *M*. IPA, 4-imidazolone-5-propionate; FGA, *N*-forminimo-L-glutamate.

tracts were prepared and assayed for enzyme activity as described (10, 11). A modified procedure for the assay of H and G enzymes in suspensions of whole cells was used. Cells were harvested by centrifugation and washed once with one half the original volume of buffer (potassium phosphate, pH 7.4, 0.01 M, containing 5 mM 2-mercaptoethanol), and resuspended in one-tenth the original volume of the same buffer. The assay procedures were as described (11), except that CTAB (hexadecyltrimethylammonium bromide) was added to the assay mixture at a final concentration of 20 μg/ml. The cells were incubated at 37°C in the CTAB-buffer mixture for 10-30 min before the reaction was started by the addition of substrate. The values obtained were normalized for the number of cells in the assay by determination of the absorbance at 277 nm of an aliquot of cells suspended in diethanolamine·HCl buffer (pH 9.4, 0.067 M) containing 20 μg/ml of CTAB. One A_{277} unit corresponds to approximately 0.05 mg of protein per ml. Specific activities are defined in the tables.

RESULTS

Evidence that the *hutC* gene product is a protein

We show here that a mutation in the *hutC* gene is subject to amber suppression. Strains bearing the *hutC7* allele express both *hut* operons constitutively (10). To determine the susceptibility of this allele to amber suppression we constructed nearly isogenic strains by transferring F′ *hut*$^+$ and F′ *hutC7* episomes from *S. typhimurium* to *E. coli* strain MS6040 (*suIII*$^+$) and as control to *E. coli* strain MS6041 (Su$^-$). The specific activity of H enzyme (coded by the *H* gene)[§] formed by these strains was measured after growth in the presence or absence of histidine as inducer. All *E. coli* strains we have examined lack all *hut* genes and enzyme activities. Therefore, all Hut enzyme activities in these hybrids come from the *S. typhimurium* genes carried on the F′ *hut* episome.

The critical data (Table 1) are the specific activities of H enzyme produced by the Su$^-$ *hutC7* strain (GS171) and by the *suIII*$^+$ *hutC7* strain (GS169) grown in the absence of inducer. Under this condition, the *suIII*$^+$ strain produces only about 10-15% as much enzyme as the nearly isogenic Su$^-$ strain. Thus, the suppressor has restored activity to the *C* gene, which is then able to repress the *H* gene. Since amber suppressors act at the level of translation (12), this result indicates that the product of the *C* gene is a protein.

Evidence that the *hutC* gene product is a repressor

We have shown that the *C*$^+$ allele is dominant to *C7* and other constitutive alleles of the *C* gene (7). This observation strongly suggests that the product of the *C*$^+$ gene is an active molecule which represses the *hut* operons, and that the product of the *C*$^-$ gene is an inactive molecule lacking the ability to repress. Another possible interpretation is that the *C*$^+$ gene product is an activator, which promotes expression of the *hut* operons only in the presence of inducer, while the *C*$^-$ gene product is

TABLE 1. *Suppression of* hutC7 *by amber suppressor* suIII

Strain	Relevant genotype	H enzyme, specific activity: Uninduced Expt. 1	Uninduced Expt. 2	Induced Expt. 1	Induced Expt. 2
GS171	*su*$^-$, *hutC7*	6.4	2.6	2.9	2.7
GS169	*suIII*$^+$, *hutC7*	0.71	0.38	4.0	3.9
GS172	*su*$^-$, *hut*$^+$	<0.05	—	2.4	—
GS170	*suIII*$^+$, *hut*$^+$	<0.05	—	3.3	—

The strains were constructed as described (7) by transferring F′ *gal*$^+$*hut*$^+$*bio*$^+$ (from NE324) or F′ *gal*$^+$*hutC7 bio*$^+$ (from NE301) to MS6041 (*gal*$^-$ Su$^-$) or to MS6040 (*gal*$^-$ *suIII*$^+$) by selecting Gal$^+$ recombinants.

The cells were grown in succinate-ammonia minimal medium to which was added 0.2% L-histidine as inducer where indicated. H enzyme activity was determined in whole-cell suspensions to which CTAB was added as described in *Methods*. Specific activity is expressed as nanomoles of product formed per minute per A_{277} unit.

§ Symbols in roman type indicate enzymes or phenotype, while symbols in italic type indicate genes or genotype.

TABLE 2. *Constitutive enzyme synthesis in strains carrying deletions of the* C *gene*

Strain	*hut* genotype	Specific activity	
		I enzyme	G enzyme
MR134	Non-lysogen, hut^-	<0.2	<1
GS139	Intact *hut* region	3.6	70
" *	"	17	233
GS185	Deletion 102	22	<1
GS187	Deletion 113	15	<1
GS188	Deletion 115	12	<1
GS189	Deletion 119	18	<1
GS190	Deletion 120	7.4	<1
GS191	Deletion 136	33	<1
GS192	Deletion 139	14	<1
GS193	Deletion 142	14	<1
GS194	Deletion 146	17	<1
GS195	Deletion 161	15	<1

Fig. 1 shows the extent of these deletions, all of which are class B. Strains with prefix GS are λ*phut* lysogens of MR134. By itself, MR134 carries no detectable *hut* genes (8). The *hut* genes, when present, are located on λ*phut* bearing the deletion indicated.

The cells were grown in succinate–ammonia minimal medium. Enzyme activities were determined in cell-free extracts as described (11). Specific activity is expressed as nanomoles of substrate consumed or product formed per min per mg of protein.

* This culture was induced with 0.2% histidine.

TABLE 3. *Decreased specific activities of U and H enzymes in* hutM *mutants*

Enzyme	Glucose addition	Strain, *hut* genotype			
		NE402 M^+,*R9*	NE433 *M139,R9*	NE445 F'M^+,*R9*	NE446 F'*M145,R9*
		Specific activity			
I	−	18,17,16	35,35	34	170
G	−	520,310,340	1800,850	720	2600
U	−	92,88,97	71,79	130	55
H	−	510,450,450	420,450	740	400
I	+	6.4	14	17	150
G	+	180	320	230	2100
U	+	11	3.3	25	4.6
H	+	130	73	200	100

Strains NE402 and NE433 are nearly isogenic strains derived uy a series of transductions with phage P376 into strain NE338 (*gal-814, bio-25*) (7, 11). These strains carry *bio-25* and are F^-. Strains NE445 and NE446 carry the indicated F' *hut* episome and the *hut*162* deletion which removes all *hut* genes from the chromosome. Strain NE445 was derived similarly to NE446 whose genealogy is given in ref. 7.

Procedures for the growth of cells, preparation of cell free extracts, assay of enzymes and specific activity units are given in Table 2. Glucose, 0.4%, was added to produce catabolite repression where indicated. All cultures contained 0.2% histidine as inducer.

"superactive," having gained the ability to promote expression even in the absence of inducer. The dominance of C^+ to C^- must then be explained by subunit mixing of C^+ and C^- gene products to produce a molecule requiring inducer for activity. Here we eliminate this possibility by showing that deletion of the *hutC* gene results in constitutive expression of *hut*.

Deletion mutations in the *hut* region have been isolated by the use of specialized transducing phages. From a *Salmonella–Escherichia* hybrid having the gene order *gal att*λ *hutM I G C (P,R) Q U H bio*, we have isolated λ transducing phages carrying *hut* genes (8). These phages carry the *M* promoter, at least part of the *I* gene, and various lengths of the *hut* region extending to the right of *I*. We have examined the specific activity of I enzyme in cells lysogenic for phages carrying only the *MI* segment (that is, lacking the *G C (P,R) Q U H* genes).

As shown in Table 2, the parent *E. coli* strain MR134, nonlysogenic for λ*phut* and hence devoid of *hut* genes, has no detectable I or G enzyme activity. Strain GS139, lysogenic for a λ*phut* phage carrying the entire *hut* region, produces both of these enzymes, whose levels are induced about 4-fold by histidine. None of the strains lysogenic for λ*phut* phages carrying only the *MI* part of the *hut* region produce any detectable G enzyme. This is the expected result, since they lack part of the *G* gene. However, all of the lysogens produce I enzyme in the absence of inducer at a level approximately equal to that of strain GS139 grown in the presence of inducer. Thus, the deletions allow full, constitutive expression of the *I* gene. We conclude therefore that the *C* gene product is not required for expression of the *hut* genes. (We assume that deletion *only* of the *C* gene would result in constitutive expression of all four *hut* genes, just as the presence of the amber *C7* mutation does. Such a strain is not available for this determination.)

Evidence that the *M* promoter governs *C* gene expression

Here we report that *M* promoter "up" mutants appear to have increased levels of repressor. We previously described mutations in *hutM*, the promoter of the left-hand operon, which act in *cis* position to increase 2- to 5-fold the level of expression of *I* and *G*, the structural genes of the left-hand operon (11). These "promoter-up" mutations were obtained by selecting mutants able to grow rapidly in a medium containing histidine as sole source of carbon. This apparently selects for mutants with increased I and (or) G enzyme activities.

In Table 3 we report measurements of all four Hut enzyme activities in strains carrying either wild-type or mutant *M* alleles. The presence of the *M139* or *M145* allele consistently decreases the activities of U and H enzymes (right-hand operon). Thus, *M139*, which increases about 2-fold the induced activities of the I and G enzymes (left-hand operon), decreases by about 20% the activities of the U and H enzymes (right-hand operon); *M145*, which increases about 5-fold the activities of the enzymes from the left-hand operon, decreases by about 50% the activities of the enzymes from the right-hand operon. (In separate experiments, we showed that the greater effects of *M145*, relative to *M139*, are also found when the *hut* genes are located on the chromosome, as well as when they are located on an episome as reported in Table 3.) For reasons discussed later, we compared enzyme activities in strains containing the *hutR9* allele, which renders the *UH* operon partially resistant to catabolite repression. It should be noted here that the residual sensitivity to catabolite repression in strains bearing the *R9* allele, combined with the apparent "repression" by *M139* or *M145*, results in very low concentrations of U and H enzymes when the strains are grown in the presence of glucose.

TABLE 4. *Enzyme levels in derivatives of* hutM *mutants*

Strain	Genotype	Specific activity			
		Uninduced		Induced	
		G enzyme	H enzyme	G enzyme	H enzyme
Parents:					
NE2	*hutR9*	2.2	1.1	2.3	14
NE385	*hutM145, R9*	12	0.3	31	7.3
Pseudorevertants of NE385:					
NE421	*hutM145, R9, C151*	54	31	21	14
NE422	*hutM145, R9, C152*	52	31	18	15
NE424	*hutM145, R9, C154*	46	25	19	14
NE423	*hutM145, R9, Q153*	5.4	6.5	17	14
NE426	*hutM145, R9, Q156*	10	6.6	28	13
Transductants:					
NE513	*hutM145, R9, C7*	55	39	12	15
NE514	*hutM145, R9, C7*	58	34	11	15

Strains NE2 and NE385 have been described (10, 11). Strains NE421 to NE424 and NE426 are spontaneous mutants of NE385 selected for their ability to grow on glucose-histidine. Strains NE513 and NE514 were obtained by transduction of NE404 (*hutM145, R9, bio-25*) to Bio$^+$ with phage P376 grown on NE464 (*hutC7, R9*), which was ultimately derived from strains NE2 (*hutR9*) and NE7 (*hutC7*) by transduction and episome transfer.

Procedures for the growth of cells and assay of enzymes, and specific activity units are given in Table 1.

TABLE 5. *Effect of deletion of M promoter on expression of "right-hand" operon*

Strain	*hut* genotype	Inducer	Specific activity	
			U enzyme	H enzyme
GS163	F′ M^+, $C7$, $U25$, H^+	−	0.36	4.7
	λ $M^-(del)$, C^+, U^+, $H1$	+	0.56	5.3
GS203	F′ M^+, $C7$, $U25$, H^+	−	0.04	1.6
	λ M^+, C^+, U^+, $H1$	+	0.42	5.5
GS212	F′ M^+, C^+, $U160$, H^+	−	0.02	0.4
	λ $M^-(del)$, C^+, U^+, $H1$	+	0.34	3.8
GS204	F′ M^+, C^+, $U160$, H^+	−	0.02	1.1
	λ M^+, C^+, U^+, $H1$	+	0.27	4.5

The strains were constructed as described (8). Strain GS67 (F′ *hutH1*) was infected with phages carrying normal *hutM* or deleted *hutM*, class 9; the recombinant phages carrying *hutH1* were used to lysogenize strain MR134; into these lysogens we introduced F′*hutC*$^+$*U160* or F′ *hutC7, U25* episomes from strains NE479 and NE438, respectively. Procedures for the growth of cells and assay of enzymes and specific activity units are given in Table 1.

We propose that the *M* promoter mutations result in decreased levels of U and H enzyme by increasing the concentration of repressor. If this interpretation is correct, restoration of high levels of enzymes should accompany abolition of repression. That is, constitutive mutants should be found among pseudorevertants of *M* mutants selected for higher activities of U and H enzymes. Likewise, strains made constitutive by transduction should have regained the ability to synthesize the enzymes at a high rate. These expectations were verified as follows.

The *hutM* mutants discussed here were originally derived from strain NE2, which carries a promoter mutation (*hutR9*) rendering the *UH* operon partially resistant to catabolite repression and thus allowing the organism to grow on a medium containing glucose as principal carbon source and histidine as sole nitrogen source (11). Unlike their parent NE2 (M^+, *R9*), the mutants NE363 (*M139, R9*) and NE385 (*M145, R9*) grow poorly on glucose–histidine medium and are thus phenotypically R^+. That these mutants NE363 and NE385 are still genotypically *R9* was demonstrated by recovering, through transduction into M^+R^+ recipients, strains that had the original HutR$^-$ phenotype. The HutR$^+$ phenotype of NE363 and NE385 presumably results from their decreased levels of U and H enzymes relative to the parental strain NE2 (see Table 3). (For the experiments reported in Table 3, we used nearly isogenic strains derived from the original mutants. NE402 and NE445 are comparable to NE2; NE433, to NE363; and NE446, to NE385.)

We examined 67 spontaneous mutants of strain NE385 (*M145, R9*) selected for their ability to grow well on glucose–histidine medium. None were true revertants of *M145*, since all had retained the ability to grow on a medium containing histidine as sole source of carbon and nitrogen, the phenotype for which the *hutM* mutants had been selected. Unlike strain NE385, however, all of these pseudorevertants produced H enzyme constitutively, as determined by the qualitative spot assay (9). Five of these mutants were analyzed further (see Table 4). Three strains—NE421, NE422, and NE424—have the HutC$^-$ phenotype. In the non-inducing medium, they produce high activities of H enzyme (right-hand operon) and G enzyme (left-hand operon). Two other strains, NE423 and NE426, have the phenotype expected of mutants with an operator–constitutive mutation in the right-hand operon. They have a higher uninduced level of H enzyme (right-hand operon) but not of G enzyme (left-hand operon). Under induced conditions, all of the mutants, like strain NE2, produce about twice as much H enzyme as the parent strain NE385.

We replaced by transduction the C^+ gene in an *M* mutant with the constitutive *C7* allele. The specific activities of H enzyme in two *hutM145, C7, R9* recombinants, strains NE513 and NE514, are twice as high as that in the parent, NE385 (*M145, R9*) (see Table 4). These results support the hypothesis that the mutation in the *M* promoter (*M145*) has increased the concentration of repressor.

Deletions removing *hutM* and abolishing repression

We can account for the increased concentration of repressor in the *M* promoter mutants by assuming that the *C* gene is part of the operon that has *M* as its promoter (left-hand operon). Deletions removing *hutM* should therefore abolish expression of the *C* gene.

We have described the isolation of λ *phut* transducing phages that carry deletions which enter the *hut* region from the left (8). Of interest here are those phages that have lost the *M* promoter but have retained the *C(P,R)QUH* genes. The extents of these deletions are shown in Fig. 1. We determined the activity of H enzyme (right-hand operon) in two independent lysogens of four class 9 deletion phages and

of two class 8 deletion phages. All of these strains had the C^- phenotype (9).

For the detailed analysis of *hutC* gene expression we constructed strain GS163 (Table 5). In this strain the λ *phut* carries a class 9 deletion (see Fig. 1) and a defective *H* gene; the introduced F'*hut* episome carries the *C7* constitutive allele and has a defective *U* gene. As shown in Table 5, H enzyme synthesis in this strain is constitutive. Apparently, the λ*phut*, though carrying an intact *C* gene, does not produce the *trans*-active repressor coded by the *C* gene. The other experiments in Table 5 are controls. They demonstrate that a λ*phut* with intact *M* produces the repressor (strain GS203) and that the episomal *C* gene product is an effective repressor of the *U* gene carried by the λ *phut* with the class 9 deletion (strain GS212).

DISCUSSION

We have previously shown that mutations in the *hutC* gene result in constitutive expression of the two *hut* operons; that the C^+, inducible allele is dominant to the C^-, constitutive alleles, and that the product of the C^+ gene acts on the *hut* operons in *trans* (7). We have now shown that one of the *hutC*$^-$ mutations is amber; therefore the active product of the *C* gene is a protein. We have also shown that deletion of the *C* gene results in constitutive expression of the remaining *hut* operon; therefore the protein coded by the *C* gene is a repressor not required for the expression of the operon.

The investigation of *hutM* mutants with increased level of expression of the left-hand operon has led us to conclude that these mutants contain an increased concentration of the repressor determined by the *C* gene (see Tables 3 and 4). Apparently the *C* gene is part of the left-hand operon (see Fig. 1). This concept is supported by the finding that a deletion of *M* that leaves *C* intact (see Fig. 1) results in the inability to produce the repressor (see Table 5). Thus, *M* appears to be the promoter for the left-hand operon consisting of the structural genes *I*, *G*, and *C*.

The repressor determined by the *C* gene acts on both operons. The operator for the right-hand operon, *hutQ*, has been identified (7). Presumably an operator for the left-hand operon is located near *hutM*. By acting on this operator the repressor represses its own synthesis. The left-hand operon, *MIGC*, is thus self-regulating. If an excess of repressor accumulates, expression of this operon and therefore of *C* will be repressed until the appropriate concentration of repressor is achieved by dilution during growth of the cell. On the other hand, if a deficiency of repressor exists, the operon will be derepressed until the concentration of repressor is increased to the steady-state level.

The proper induction of the *hut* system requires both operons. We have just noted that the left-hand operon produces the repressor. We have previously shown that the right-hand operon produces the inducer. Histidine, the compound whose presence in the growth medium brings about induction of the system, must be converted to the actual inducer (urocanate) by histidase, the product of the *H* gene (9, 10). Urocanase, the product of the *U* gene, destroys the inducer. The fact that histidase and urocanase are coordinately controlled as products of the same operon insures a a proper ratio of these enzyme activities and thus a concentration of urocanate adequate for induction. However, urocanate in turn induces the formation of the repressor; thus, a supply of histidine adequate to maintain a high enough intracellular level of urocanate to inactivate the induced repressor is required for full induction of both operons.

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