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Regulation of the Histidine Utilization (Hut) System in Bacteria

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INTRODUCTION

The ability to degrade the amino acid histidine to ammonia, glutamate, and a one-carbon compound (formate or formamide) is a property that is widely distributed among bacteria (62). It was the study of this pathway's regulation in the Gram-negative bacteria that led to the discovery of three important regulatory paradigms: carbon catabolite repression (87), the two-component Ntr (nitrogen regulatory) system (88, 121), and autogenous regulation of transcriptional regulators (149). Similarly, the study of Hut regulation in the Gram-positive bacterium *Bacillus subtilis* was one of the routes that led to the discovery of CodY, an extraordinary regulatory protein that integrates signals reflecting protein synthesis, nucleic acid synthesis, and energy availability to control a wide variety of responses (151). Yet there has been only one review of the histidine utilization (Hut) system (62) since those of Tabor in 1954 (153) and Magasanik in 1978 (89). The principal focus of this review is the regulation of the Hut pathway. Therefore, the *hut* system of *Klebsiella pneumoniae* (formerly known as *Klebsiella aerogenes* and also as *Aerobacter aerogenes*) serves as a reference point for much of this discussion, because the regulation is best characterized for this organism. The other foci are the *hut* system of *Bacillus subtilis*, whose regulatory mechanisms are very

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doi:10.1128/MMBR.00014-12

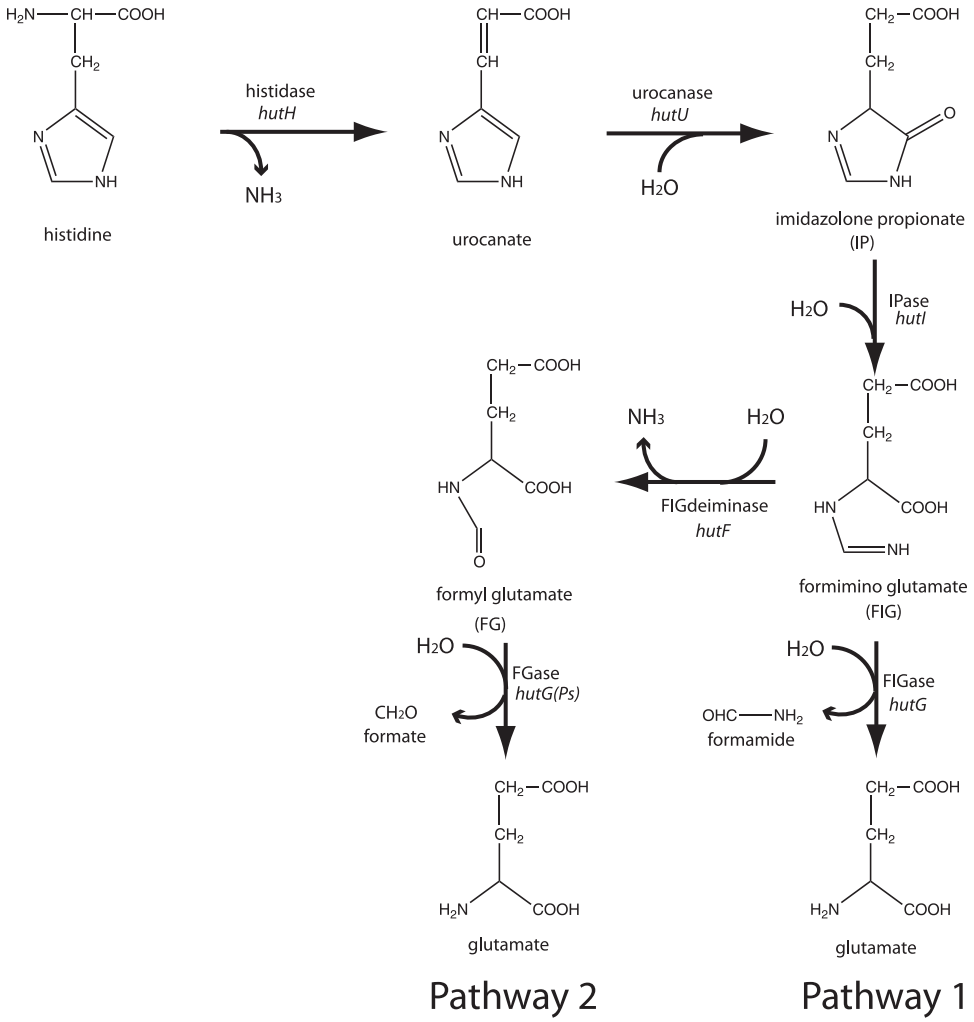

FIG 1 The two conserved histidine utilization (Hut) pathways. Pathway 1 yields 1 mole each of ammonia, glutamate, and formamide per mole of histidine. Pathway 2 yields 2 moles of ammonia, 1 mole of glutamate, and 1 mole of formate per mole of histidine.

different, and the *hut* system of the pseudomonads, whose enzymology is most thoroughly characterized.

THE HUT PATHWAY

The pathway of histidine catabolism (Fig. 1) is highly conserved among bacteria. The first three steps appear to be universal. They involve the elimination of ammonia from histidine to yield urocanate, hydration of urocanate to give imidazolone propionate (IP), and ring cleavage of IP to yield formiminoglutamate (FIG). There are two different fates for FIG. In some genera (e.g., *Klebsiella* and *Bacillus*), FIG is hydrolyzed to formamide and glutamate, with the formamide being excreted as a waste product (67, 90). In other genera (e.g., *Pseudomonas* and *Streptomyces*), the imino group of FIG is first hydrolyzed to yield ammonia and formylglutamate (FG). FG is then hydrolyzed to give formate and glutamate (70, 154). The two pathways are easily distinguished by growth tests in most cases. In pathway 1, 1 mole of histidine yields 2 moles of useable nitrogen (1 mole as ammonia and 1 mole as glutamate). In pathway 2, 1 mole of histidine yields three useable nitrogen atoms (Fig. 1). Thus, when organisms are fed limiting amounts of histidine as the sole nitrogen source, those with pathway 1 will give a final growth yield per mole of histidine that is twice that per mole of ammonia, and those with pathway 2 will give three times the yield per mole of histidine than per mole of ammonia. However, the Hut system of *Caulobacter crescentus* provides an exception where the growth test result is in conflict with the actual pathway (see below).

THE HUT ENZYMES

Histidase

Histidase, the first enzyme in the pathway, is widely distributed among the bacterial and eukaryotic domains. It is highly conserved (Table 1), with >40% amino acid identity between the bacterial and mammalian enzymes (157). Purified histidase is a tetramer of four identical subunits, with just over 500 amino acids in each subunit (54). The chemistry of the histidase reaction is rather unusual. The catabolism of many amino acids begins with the conversion of the amino acid to the corresponding keto acid, either by transamination or by oxidative deamination (99). In contrast, histidase catalyzes a nonoxidative reaction that liberates the amino group, yielding urocanate as the first intermediate in

TABLE 1 Conservation of Hut enzyme sequences

Enzyme[a]	% Amino acid sequence identity/% amino acid similarity[b]				
	Salmonella	*Pseudomonas*	*Bacillus*	*Streptomyces*	Human
HutH (histidase)	82/90	79/85	44/63	44/60	43/60
HutU (urocanase)	89/95	86/92	63/79	59/72	35/53
HutI (IPase)	70/83	35/69	40/70	36/50 (39/51)	34/51
HutG (FIGase)	67/75	NA	23/46	NA	NA
P. putida HutF				(36/52)	
P. putida HutG(Ps)				(24/38)	

[a] Unless noted otherwise, the enzymes are from *Klebsiella pneumoniae*, and the protein sequences of the other organisms are compared to them.
[b] Determined by a BLAST search. The numbers in parentheses represent comparison to the corresponding *Pseudomonas putida* sequence. NA, not applicable.

the pathway (90, 156). This reaction requires the presence of a strong electrophile at the active site of the enzyme. Despite an early belief that this electrophile was a dehydroalanine residue generated by the dehydration of a serine (Ser143) within the active site of the *Pseudomonas* enzyme (41, 163), direct evidence was lacking and early experiments were inconsistent with the presence of dehydroalanine (55). The three-dimensional structure of the *Pseudomonas* histidase revealed a novel imidazole structure, 4-methylidene-imidazole-5-one (MIO), generated from cyclization of residues 142 to 144 (143). This MIO structure (Fig. 2) allows the enzyme to remove a nonacidic proton from the β carbon atom while keeping the protonation of the leaving amino group on the adjacent α carbon (132, 143). The product of this α-β elimination reaction is *trans*-urocanate, a strongly UV-absorbing compound whose unusual name reflects the fact that it was originally isolated from the urine of a dog (63). Bacteria lacking histidase display no phenotype other than the inability to use histidine as a carbon source. Mammals lacking histidase suffer from histidinemia (158).

Urocanase

Urocanase, the second enzyme in the pathway, is a homodimer with 557 amino acids in each subunit of the *Pseudomonas* enzyme (72, 83). Like histidase, it is widely distributed within the bacterial domain, though less so in the archaeal and eukaryotic domains. The amino acid sequence is highly conserved (29, 76). For example, the urocanase of *Pseudomonas* shares about 35% identity with the human UROC1 gene product over the 500-amino-acid stretch corresponding to the bacterial enzyme (Table 1). Surprisingly, it shows more than 70% identity with the urocanase enzyme from

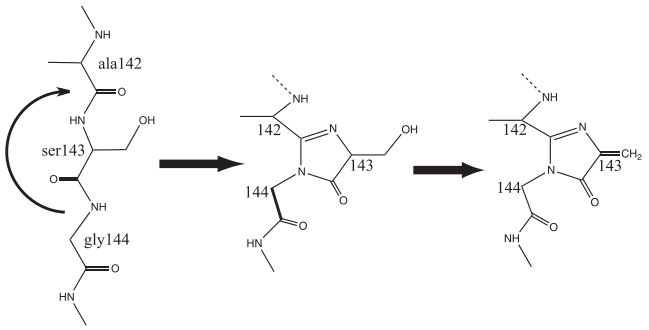

FIG 2 Formation of MIO, an unusual imidazole structure generated at the active site of histidase, as described by Schwede et al. (143).

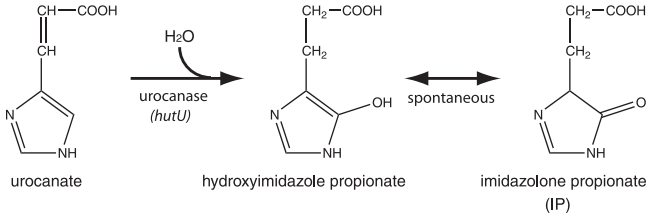

FIG 3 Urocanase reaction. Hydration of the C═C double bond of urocanate generates hydroxyimidazole propionate, which spontaneously undergoes an enol-keto tautomerization to imidazolone propionate (IP).

plants (76), suggesting horizontal transfer. Each monomer of urocanase contains one molecule of tightly bound NAD^+, which is required for its activity (27, 127). The crystal structure of urocanase (72) has confirmed the unprecedented role of the tightly bound NAD^+ in the reaction mechanism, where it serves as an electrophile rather than as a redox center. The electrophilic attack on the C═C double bond of the imidazole ring results in the hydration of the intermediate and the production of hydroxyimidazole propionate, which in turn generates imidazolone propionate by an enol-keto tautomerization (Fig. 3) (96, 128).

IPase

Imidazolone propionate hydrolase (IPase), the third enzyme in the pathway, is a homodimeric metalloenzyme with about 400 to 450 amino acids per monomer, depending on the source of the enzyme. The enzyme from *Bacillus subtilis* has 421 amino acids per monomer (170), which is slightly larger than the predicted *Pseudomonas* enzyme. Its substrate, IP, is unstable, with a half-life of about 20 to 25 min (125, 128). Early kinetic studies used IP produced *in situ* from urocanate in the presence of highly purified urocanase. As a result, the characterization of IPase lagged behind that of the other Hut enzymes, and IPase was the last of the enzymes to have its crystal structure determined (161, 170). Although the details of the reaction mechanism and the nature of the active site metal remain somewhat unclear, two facts are well established: the reaction is essential for utilization of histidine as a carbon source (74, 148), and IPase is a hydrolase that cleaves the ring to yield formiminoglutamate, in a different reaction from the nonenzymatic cleavage that yields formylisoglutamine (128). It is interesting that mutants lacking IPase are poisoned by growth in the presence of histidine if histidase and urocanase are both active (14, 48). The reason for this toxicity is unknown but might result from accumulation of formylisoglutamine or 4-ketoglutaramic acid derived from the spontaneous decomposition of IP (97, 128). That would be consistent with the observation that either a very high concentration of exogenous histidine (48) or a large amount of histidase and urocanase activity (14) is required for histidine to inhibit growth effectively. It has also been suggested that both imidazole propionate and IP or products derived *in vivo* from them may somehow interfere with the aspartate aminotransferase of *Salmonella enterica* and that this might explain the toxicity of IP (12). However, neither imidazole propionate nor IP inhibits aspartate aminotransferase *in vitro* (12), so a mechanism for this effect remains unclear. In any event, it is clear that accumulation of intermediates of the Hut pathway, their analogs, or their metabolites can have deleterious effects on growth. Careful regulation of the pathway is thus essential.

The first three enzymes in the pathway of histidine utilization

are the same in all genera that have a Hut pathway. But there are two different fates of FIG (Fig. 1), depending on the genus of the bacterium. In pathway 1, formamide is liberated from FIG, leaving glutamate, with the formamide as a waste product (90). In pathway 2, ammonia is first liberated from FIG and then the resulting FG is hydrolyzed to give glutamate and formate (154). These last steps (three enzymes) are less well understood.

FIGase

In pathway 1 (Fig. 1), as characterized for the enteric bacteria and *B. subtilis*, IP is cleaved to glutamate and formamide and the formamide is not further catabolized. Under aerobic conditions, the glutamate is then further catabolized to yield both a source of carbon and energy and a second nitrogen. Formiminoglutamate hydrolase (FIGase), the last enzyme in this pathway, is less well characterized than the others. The mass of native FIGase from *B. subtilis* was estimated to be about 220 kDa by sedimentation velocity ultracentrifugation (67). The subunit mass calculated from the sequence of the *hutG* gene is about 35 kDa, suggesting that FIGase is a hexamer of identical subunits. The crystal structure of the FIGase from *B. subtilis* has been deposited in the Protein Data Bank (PDB; accession number 1XFK), but no analysis derived from this structure has yet appeared. The *Bacillus* enzyme, like that from *Klebsiella*, requires a manganese cofactor and appears to have a very low affinity (K_m of about 40 or 50 mM) for the substrate FIG (67, 82). It is interesting that there is considerably more sequence divergence between the *Bacillus* and *Klebsiella* FIGases than that seen with the other three enzymes of the pathway (Table 1). Even the FIGase from *Salmonella*, a close relative of *Klebsiella*, is more divergent than might be expected. FIGase is closely related to a family of arginase enzymes, leading one to speculate that FIGase may be a relatively recent addition to the pathway and that it is not yet fully adapted to its role in histidine utilization. Moreover, the relatively high apparent K_m of FIGase for FIG, if truly representative of the condition *in vivo*, leads one to wonder whether the intracellular concentration of FIG ever reaches this high level or whether some other feature (metabolite channeling?) might drive this reaction.

FIG Deiminase

In pathway 2, found in *Pseudomonas* and other genera, FIG is first hydrolyzed to formylglutamate and ammonia, thus liberating a second molecule of ammonia from each molecule of histidine (164). The resulting formylglutamate is then hydrolyzed to formate and glutamate, both of which can be used for biosynthesis (155). These two reactions are carried out by the products of the *hutF* and *hutG* genes, respectively, of *Pseudomonas* (58). Because of the confusion between the *hutG* gene of *Pseudomonas* and the unrelated *hutG* gene of *Klebsiella*, I qualify the HutG enzyme from pathway 2 as HutG(Ps) from here on. A recent report found an activity in *Pseudomonas aeruginosa* that was capable of carrying out the pathway 1 reaction (FIGase) *in vitro* (95), but genetic analysis has confirmed that this activity (whatever it is) cannot replace HutF and HutG(Ps) to allow histidine to be used as a carbon source (175). Thus, the two pathways appear to be mutually exclusive.

FIG deiminase (the HutF enzyme of *Pseudomonas*) is a homodimer with about 450 amino acids per monomer (94, 164). The enzyme contains one atom of zinc per monomer, which is required for its activity (94). FIG deiminase is a member of the aminohydrolase superfamily and shares many properties with other members of the family, especially around the active site (94). FIG deiminase has about a 10-fold higher affinity for FIG than the FIGase of pathway 1, with a K_m of about 220 μM for FIG deiminase (94) versus 40 to 50 mM for FIGase (67, 82). This suggests that the HutF enzyme found in pathway 2 may be better adapted to its role in histidine utilization than the HutG enzyme found in pathway 1.

TABLE 2 Conservation of HutT gene orthologs

Protein (species)[a]	% Amino acid sequence identity/% amino acid similarity[b]			
	ProY (*Ppu*)	PA5097 (*Pae*)	ProY (*Kpn*)	HutM (*Bsu*)
HutT (*Pfl*)	85/92	78/88	60/78	35/56
ProY (*Ppu*)		76/85	59/76	36/57
PA5097 (*Pae*)			62/79	36/58
ProY (*Kpn*)				36/58

[a] HutT of *P. fluorescens* has been shown to be a histidine transporter (129, 174); it is not known whether it also transports urocanate, but it seems likely that it does. The other proteins in the first column are orthologous, encoded by part of the *hut* operon in the corresponding species, and almost certainly homologous to *hutT*. Note that ProY (indicated as HutT in Fig. 4) of *K. pneumoniae* is a urocanate transporter (136). Species designations: *Pfl*, *P. fluorescens*; *Ppu*, *P. putida*; *Pae*, *P. aeruginosa*; *Kpn*, *K. pneumoniae*; *Bsu*, *B. subtilis*.

[b] Determined by a BLAST search.

FGase

The last step in pathway 2 is the hydrolysis of FG to glutamate and formate. The formylglutamate hydrolase (FGase) of *Pseudomonas* appears to be a monomer of about 50 kDa (about 460 amino acids) and to be stimulated by divalent cations, particularly Co^{2+} and Fe^{2+} (57). The relatively high K_m of the enzyme for FG (about 12 mM) may suggest that this enzyme is not native to the pathway but was recruited from a family of hydrolases and then adapted to allow the cell to derive glutamate from histidine degradation. The overlapping specificities of these related hydrolases may explain the difficulty in isolating *hutG* mutants of *Pseudomonas putida* (57). Nevertheless, *hutG* is clearly part of the *hut* operon of *P. putida*, and plasmids with an insertion in *hutG* are unable to confer histidine utilization on *Escherichia coli* (58). Thus, FGase is certainly part of pathway 2 (Fig. 1). The absence of a crystal structure for the enzyme leaves a gap in the understanding of this pathway. An enzyme capable of cleaving FG has been found in mammals, but this appears to be an unrelated protein (102).

Histidine and Urocanate Permeases

The transport components of the Hut system are the least well-characterized part of the picture. Structural and genetic studies of histidine and urocanate transporters are lacking. Bacteria appear to have a variety of permeases capable of transporting histidine, and these vary in affinity, capacity, and regulation (3). Partly because of this, early genetic screens yielded no *hut* mutants that were defective in a Hut-specific histidine transporter (165), nor have any urocanate transport mutants been identified or mapped. Nevertheless, it appears that most clusters of *hut* genes contain at least one transporter for histidine or urocanate, often mistakenly annotated ProY (a proline transporter) (Table 2). The *Pseudomonas fluorescens hut* cluster (Fig. 4) contains a gene annotated *hutT* which is required for the growth of *E. coli* with histidine as the sole carbon source (174). Clones containing the entire *hut* region from *P. fluorescens* confer this ability on *E. coli*, but clones in which *hutT*

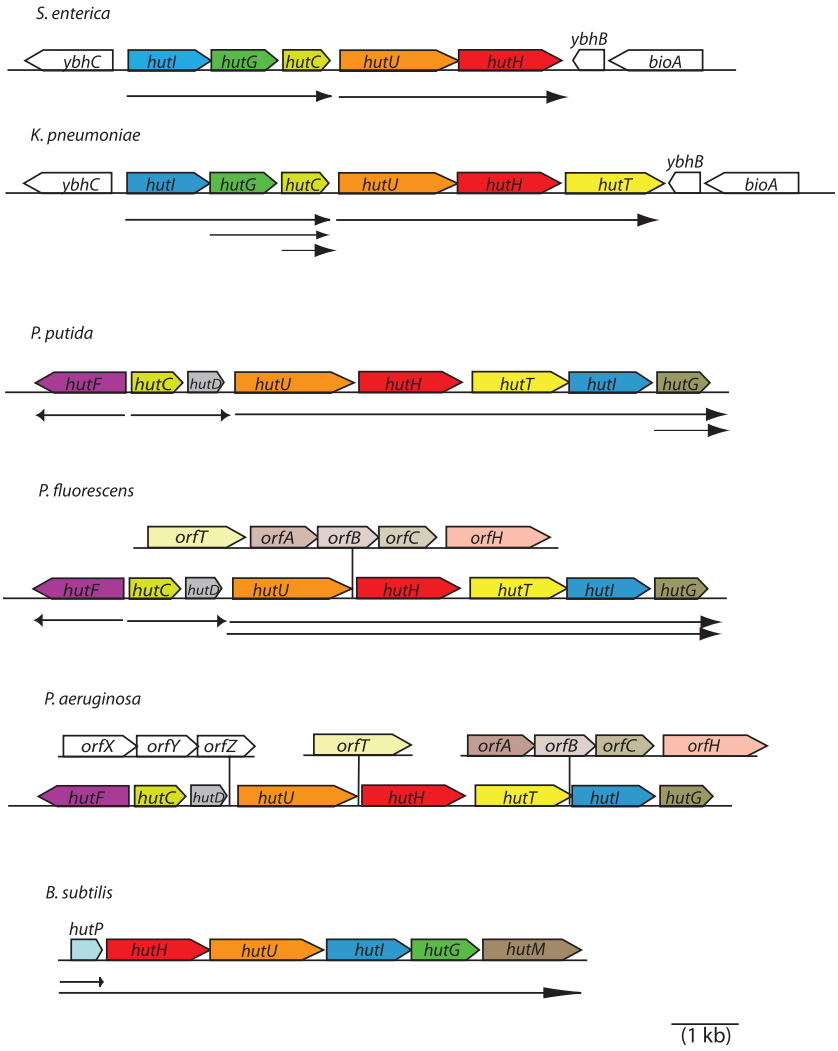

FIG 4 Genetic structure of the *hut* operons. Genes encoding elements of the Hut pathway are shown on the main line and in bold colors. Genes with the same name and same color are homologous. [Note that *hutG* of the enteric bacteria is not homologous to *hutG*(*Ps*) of the pseudomonads.] Genes of unknown function that are not part of the *hut* pathway are listed with *orf* names rather than *hut* names and appear on lines above the main line. *orfH* (sometimes called *hutH1*) is similar to *hutH*, but its gene product lacks histidase activity. *orfT* is similar to *hutT*, but its gene product cannot replace the *hutT* gene product for transporting histidine or urocanate. *orfA*, *-B*, *-C*, *-D*, *-H*, and *-T* may have functions that are related to histidine degradation (see the text) and are shown in pastel colors. Genes shown without color are unrelated to *hut*. Arrows under the main line indicate transcription units and directions. The illustration was drawn approximately to scale.

has been deleted do not, even though all the enzymes of the pathway are present and expressed. Thus, HutT is a Hut-specific histidine transporter in *P. fluorescens*. Similarly, insertion mutations in the *hutT* gene of *P. aeruginosa* abolish the ability to grow with histidine as a carbon source (129). Curiously, the *P. fluorescens* and *P. aeruginosa hut* clusters (but not that of *P. putida*) also contain another putative permease gene and a cluster of 3 genes (Fig. 4) that probably encode an ABC-type transport system of unknown function (175). Genome alignment of *P. putida* with *P. fluorescens* and *P. aeruginosa* reveals conservation of the sequence and location (immediately downstream from *hutH*) of a homolog of the *hutT* gene. Its location and its orthology to *hutT* of *P. fluorescens* and *P. aeruginosa* (Table 2) argue that all of these encode Hut-specific histidine transporters as well and should be reannotated *hutT* rather than *proY*.

In *K. pneumoniae*, immediately downstream from *hutH* lies a gene that is also, by analogy, annotated *proY* (a proline specific transporter). The location of this gene and the similarity of its product to HutT (Table 2) suggest that it too may be a Hut-specific transporter gene. *K. pneumoniae* is known to have an inducible permease capable of transporting urocanate as well as the nonmetabolizable, gratuitous inducer imidazole propionate (136). This inducible transporter is not present in the closely related enteric bacterium *S. enterica* (15, 100), nor is there a corresponding open reading frame (ORF) downstream of *hutH* in *S. enterica* (Fig. 4). Nevertheless, *S. enterica* can grow well with histidine as a carbon source (15). Since *S. enterica* can still grow with histidine as a carbon source, it seems unlikely that the *hutT* gene of *K. pneumoniae* is required for histidine transport. Moreover, competition studies show that histidine is not likely to be a substrate for HutT of *K. pneumoniae* and that this enzyme is most likely the inducible urocanate transporter described by Schlesinger and Magasanik (136).

The product of the last gene in the *hut* operon of *B. subtilis*,

HutM, is 36% identical and 56% similar to the HutT enzyme of *P. fluorescens*. Although this degree of similarity is comparable to that seen with many other amino acid permeases, the location of the *hutM* gene argues that it is also a histidine transporter gene. It is not known whether *B. subtilis* has a Hut-specific urocanate transporter.

PHYLOGENETIC DISTRIBUTION OF THE HUT PATHWAY

It is clear that the Hut pathway is widespread, but it is harder to say precisely how widespread. One can use the cooccurrence of orthologs of the first three enzymes (common to both versions of the Hut pathway) as a surrogate for the presence of Hut. Using this criterion, the Web resource String (http://string.embl.de/) allows one to suggest the presence or absence of Hut in organisms whose sequenced genomes are available. A less rigorous surrogate for Hut is the single enzyme urocanase. The Web resource Pfam (http://pfam.sanger.ac.uk/) allows one to place an upper limit on the number of species with sequenced genomes that are likely to contain Hut.

Bacteria

The Hut pathway is found with high frequency in most phylogenetic groups within the bacterial domain. Pfam identifies 1,164 bacterial species with an ortholog of the urocanase gene and a similar number with a histidase gene. However, Hut is not universal within bacteria. The cyanobacteria and the green sulfur bacteria appear to lack Hut altogether. Hut also appears to be absent from the mycoplasmas, the spirochetes, and the chlamydias. The parasitic lifestyle of the latter groups may explain the loss of Hut, but the absence of Hut in the two photosynthetic groups remains unexplained. In addition to these broad generalizations about presence and absence, it is worth noting that *E. coli*, the model organism for so many studies, lacks the *hut* operons, even though its closest relatives all contain *hut*. In *E. coli*, the attachment site for phage lambda is found at the site where both *K. pneumoniae* and *S. enterica* have *hut*. Perhaps this explains the loss of *hut*. Whatever the explanation for the lack of *hut* in *E. coli*, it has made the study of this operon more difficult and less visible, despite its importance in generating critical regulatory paradigms.

Archaea

The presence of Hut in the archaea is spotty. Several thermophilic and thermoacidophilic archaea appear to have Hut, but this is by no means a common feature. The methanogens appear not to have Hut. Pfam identifies 18 archaeal species that have an ortholog of urocanase, all of which are halophiles or thermophiles. Pfam identifies many more species with an ortholog of histidase, but this probably reflects a broader family of enzymes that also include phenylalanine ammonia lyases and other ammonia lyases.

Eukaryotes

Pfam identifies 36 eukaryotic species that have an ortholog of urocanase, 21 of which are metazoans. In String, the pattern is similar. Among the lower eukaryotes, the presence of a complete Hut pathway is spotty. Among the protozoa, only *Dictyostelium discoideum* appears to have Hut. Although a variety of fungi appear to have a histidase-like enzyme (possibly a more general family of aromatic amino acid deaminases), they generally lack a recognizable ortholog of urocanase or IPase. The same is true of the entire plant kingdom, with the presence of a histidase-like protein but no recognizable urocanase. Of the lower metazoans, only the hydra seems to have a possible Hut pathway. However, the entire vertebrate branch of the evolutionary tree is replete with Hut, from echinoderms through amphibians to mammals, including mice and humans. Curiously, the arthropod branch of the tree appears to lack Hut entirely, except for a tick, which may suggest horizontal transfer from mammals. The evolutionary history of Hut in the eukaryotes presents an exciting area for investigation that is far beyond the scope of this brief discussion.

In discussing the distribution of Hut in metazoans, it is important to focus not only on which organisms contain the *hut* genes but also on which tissues of a single organism express those genes. Ever since the discovery of urocanate in the epidermis of animals (152), it has been suggested that the UV-absorbing properties of urocanic acid might make it a "natural sunscreen" for animals (152, 173). The epidermis contains histidase but no urocanase, so this sunscreen accumulates and protects against certain UV-induced DNA damage (6). However, this simplistic view is marred by the complication that irradiation of urocanate has other consequences that may negate the protective advantage (40).

hut OPERONS

Enteric Bacteria

The *hut* genes are found in many, but not all, enteric genera. For example, *Klebsiella*, *Salmonella*, *Citrobacter*, and *Enterobacter* have a *hut* cluster located adjacent to the biotin synthesis (*bio*) genes. *Vibrio* has a similar cluster, but not adjacent to *bio*. *Erwinia* also has *hut* genes, but these may be somewhat different from those of the other enterics (see the section on evolution below). However, *hut* is not universal in the enterics; for example, *Escherichia*, *Edwardsiella*, *Shigella*, and *Proteus* lack an ortholog of *hut*.

The *hut* cluster of *K. pneumoniae* is composed of six genes: the four enzyme-encoding genes, the gene for the Hut-specific repressor HutC, and the gene for the urocanate transporter HutT (Fig. 4). Genetic experiments (45) and cloning data (14) established the order of the genes to be *hutIGCUH*, the same as the order established earlier for *S. enterica* (148). Alignment of sequenced and assembled genomes by use of a simple Web-based tool (http://www.ecocyc.org/) confirms this order and places the *hut* operons immediately adjacent to the *bio* cluster. The EcoCyc tool (71) has greatly simplified the task of aligning orthologous genes and operons in the genome of one species with those in the genomes of related or unrelated species. The *hutT* gene (encoding a probable urocanate transporter) of *K. pneumoniae* lies at the end of the cluster, giving the order *hutIGCUHT-bio*. *hutT* is absent from the *hut* clusters of all other enterics, including *S. enterica*, consistent with the absence of an inducible permease capable of transporting urocanate or the gratuitous inducer of *hut* (imidazole propionate) in *S. enterica* (15, 100).

The *hutUH* genes of *K. pneumoniae* form an operon (142). Strains with insertions of Tn*1000* in *hutU* fail to make either active histidase or a HutH polypeptide. The *hutUH* operon is transcribed from a promoter located between *hutC* and *hutU* (108, 109). This is similar to the arrangement deduced by genetic analysis for the *S. enterica hutUH* genes (148). Although the *hutT* gene lies just 98 bp downstream from *hutH* in *K. pneumoniae*, it is not known whether it is part of a *hutUHT* operon, is transcribed from its own promoter, or both. The position of *hutT* relative to *hutUH* suggests a *hutUHT* operon. HutT activity is generally coregulated

with HutH, consistent with this notion (136); however, at least one datum in the initial study suggested noncoordinate regulation of HutH and HutT (136), so the existence of a separate promoter for *hutT* cannot rigorously be excluded. The *hutG* gene is not coordinately regulated with *hutH* and *hutU* (91) and thus is clearly not part of the *hutUHT* operon.

Early genetic analysis of Hut in *S. enterica* was complicated by the fact that the standard genetic strain, LT2, is phenotypically Hut negative. This strain makes low constitutive levels of histidase and undetectable levels of urocanase and does not grow with histidine as a sole nitrogen or sole carbon source (100). A mutant able to use histidine as a sole nitrogen source was obtained with difficulty after irradiation with UV light but not with several other mutagens, and genetic analysis showed that the mutation lay between a mutation in *hutC* and a mutation in *hutU* (100). Curiously, a study of *lac* fusions to the *hutUHT* promoter in *S. enterica* strains LT2 and 15-59 (Hut negative and Hut proficient, respectively) showed that the two promoters were equally active and that both were equally regulated by nitrogen when present in an *E. coli* or *K. pneumoniae* cytoplasm (unpublished observation). The DNA sequence has revealed that the defect in strain LT2 results from a −1 frameshift mutation about halfway through *hutU*: nucleotide 795 (of 1,685 nucleotides) is deleted. This explains several properties of the strain: polarity explains the low level of histidase, the excess of histidase over urocanase explains the partial constitutivity of histidase formation, and the frameshift nature of the mutation explains the failure of mutagens such as 2-aminopurine, ethyl methane sulfonate, and nitrous acid to yield Hut-proficient mutants.

Genetic analysis of *S. enterica* has established that the *hutI*, *-G*, and *-C* genes are transcribed as a single *hutIGC* operon from a promoter to the left of *hutI* (89, 149). In contrast, the *hutC* gene of *K. pneumoniae* is transcribed independently (142), as had been suspected based on physiological arguments (45). The sequence of the *hutG-hutC* intergenic region of *K. pneumoniae* shows a reasonable match to a consensus promoter (good −35 region and excellent −10 region) that extends from the end of *hutG* into the intergenic region (139). The sequence corresponding to the −10 region is missing from the shorter intergenic region in *S. enterica*, which is otherwise similar to the corresponding region from *K. pneumoniae*. A more troublesome anomaly is the fact that strains with Tn*1000* insertions in the *hutI* gene from *K. pneumoniae* still produce both HutG activity and HutG polypeptide at nearly normal levels (142). Nevertheless, the same 3-bp overlap between *hutI* and *hutG* is seen in both organisms, suggesting that *hutI* and *hutG* are probably cotranscribed. Although *hutC* is transcribed independently and *hutG* may be, it seems likely that there is an operonic *hutIGC* transcript as well, with a possible terminator or attenuator between *hutG* and *hutC* (139).

Pseudomonads

The *hut* cluster of *P. putida* was mapped by a combination of genetic and physical methods (58). The order of genes was found to be *hutF-hutC-hutU-hutH-hutI-hutG*, with *hutF* transcribed leftward and the remaining genes transcribed rightward. The original analysis showed three transcription units inducible by urocanate: *hutF*, *hutC*, and *hutUHIG*. A fourth transcription unit, inducible by urocanate and also by FG, includes only the *hutG* gene (58), reminiscent of the situation in *K. pneumoniae*. Annotation of the complete genome sequence of *P. putida* confirmed these observations and added two more genes to the cluster: *hutD* (2), a gene involved in regulation (see below); and *hutT*, a histidine transporter. This arrangement is shown in Fig. 4. Based on their close apposition (only 11-bp separation) and their induction profiles (58), it appears likely that *hutG* is transcribed both as part of a *hutUHTIG* operon (*hutU-G* operon) and as a separate gene, though the location of its promoter is unknown.

The *hut* operons of *P. fluorescens* strain SBW25 are similar to those of *P. putida*, except for an insertion of five genes between *hutU* and *hutH* (Fig. 4). The first of these encodes a putative permease whose substrate is unknown, the next three encode an apparent ABC-type transporter whose substrate is unknown, and the fifth encodes a hydrolase of unknown function whose amino acid sequence is 36% identical to HutH (175). None of these genes seem to be involved in histidine utilization. The putative permease and ABC transporter genes (*orfT*, *orfA*, *orfB*, and *orfC* in Fig. 4) cannot replace *hutT* for growth on histidine (174), and the gene with similarity to *hutH* (*orfH*) cannot replace *hutH* for growth on histidine (175). The roles of these genes are unknown, but it is significant that they also appear in the *hut* operon of *P. aeruginosa* (see below) and in other organisms, where they are not always adjacent to *hut* genes (see the section on evolution below).

The entire stretch of genes in the *P. fluorescens hut* cluster from *hutU* through *hutG* forms a single operon. In contrast to the situation in *P. putida*, where transposon insertions in *hutH* still allow expression of inducible *hutG* (58), transposon insertions in the *P. fluorescens hutU* gene abolish *hutG* expression entirely (175). The termination codon of *hutC* overlaps the initiation codon of *hutD*. This close apposition (here and in other *Pseudomonas* spp.) argues strongly that *hutC* and *hutD* form a single *hutCD* transcription unit (175). It is interesting that like the Hut systems from enteric bacteria (46, 84), all of the *hut* operons of *P. fluorescens* (and perhaps other pseudomonads [52, 64]) can be transcribed by the "housekeeping" RNA polymerase, which carries σ^{70} as its promoter recognition subunit (175, 176). However, the *hutU-G* operon of *P. fluorescens* can also be transcribed from a promoter that is recognized by the unusual RNA polymerase that carries σ^{54} (also known as σ^{N}) as its promoter recognition subunit (175, 176), as described in more detail in the section on regulation (see below).

The *hut* operons of *P. aeruginosa* also contain the same five genes as *P. fluorescens* inserted downstream of *hutU*, though they are slightly rearranged (Fig. 4). There is also another insertion of an apparent operon with three ORFs of unknown function located between the *hutCD* operon and the *hutU* operon of *P. aeruginosa*. Little is known about the transcription units of *P. aeruginosa* or other pseudomonads, but the arrangement of their operons suggests that they may be similar to those of *P. putida* and *P. fluorescens*. A general pattern is clear for the Gram-negative organisms: the *hut* genes are clustered, sometimes with another set of genes encoding a hydrolase and an ABC-type transporter, and their order is subject to rearrangements.

Bacillus subtilis

The *hut* operons of the Gram-positive organism *B. subtilis* follow this pattern of clustering, despite considerable differences in regulation of the cluster (see below). The initial genetic analysis established the order *hutHUIG*, with regulatory elements that are tightly linked and located to the left of the structural genes (74). The same study showed that the expression of the *hutH*, *hutU*,

hutI, and *hutG* gene products was coregulated, suggesting that they constitute a single *hutHUIG* operon. The regulatory elements (initially named *hutR* and *hutC*) are now understood to include a gene (*hutP*) that encodes a protein involved in antitermination of the single *hutPHUIGM* transcript and a site where HutP acts, located between *hutP* and *hutH* (114, 165, 169). The *hut* genes of *B. subtilis* lie in a single operon, in contrast to the situation in the Gram-negative organisms. However, it is interesting that there is a 3-bp overlap between *hutH* and *hutU* and a 7-bp overlap between *hutI* and *hutG*. There is a very short space (13 bp) between *hutU* and *hutI* and a longer space (76 bp) between *hutG* and *hutM*, which encodes the histidine transporter. These three groupings are reminiscent of the groupings in other organisms (see above). Although there is obvious similarity between the Hut enzymes of *B. subtilis* and those of the Gram-negative bacteria, their regulation is quite different. The inducer of the *hut* operon in *B. subtilis* is histidine, not urocanate (17), and the mode of regulation is by HutP-mediated antitermination rather than by HutC-mediated repression (114, 165, 169), as discussed below.

In contrast to *B. subtilis*, the Gram-positive organism *Streptomyces griseus* uses the five-enzyme pathway for histidine degradation (70). The *hut* genes of the streptomycetes are not well characterized, but it appears that the *hutH* gene of *Streptomyces griseus* is separate from the other *hut* genes (*hutU*, *-F*, and *-I*), which cluster with an ORF (probably *hutG*, but currently annotated as an allantoate aminohydrolase gene) in an apparent *hutU-hutG-hutF-hutI* operon.

REGULATION OF HUT EXPRESSION

Histidine is one of the most expensive amino acids in the cell, requiring an input of 20 high-energy phosphate bonds for its synthesis (1). Thus, it should not be surprising that its degradation is tightly regulated. Moreover, since a portion of the histidine biosynthetic pathway is shared with the purine biosynthetic pathway, a futile cycle of histidine synthesis and degradation would be doubly damaging. In this context, it is important to remember that histidine degradation (leading to glutamate) is not the reverse of histidine synthesis (starting with ribose phosphate). The regulation of the Hut pathway has been studied extensively in three bacterial groups: the enteric bacteria (*K. pneumoniae* and *S. enterica*), several *Pseudomonas* species (*P. putida*, *P aeruginosa*, and *P. fluorescens*), and *B. subtilis*. The details of the mechanisms differ considerably among these three groups, so they are considered separately. The three groups do, however, share several common regulatory features. (i) The Hut enzymes are not formed unless exogenous histidine is present at concentrations that exceed internal pools generated by histidine synthesis. (ii) The relative affinities of the degradative enzymes and the tRNA synthetases are such that internal pools of histidine will not be drained below a level where protein synthesis can continue. (iii) The Hut enzymes are not usually formed at maximal rates unless the cells are limited in some essential requirement that can be provided by degradation of histidine, such as a carbon source.

Enteric Bacteria

In 1952, Ushiba and Magasanik showed that a histidine auxotroph of *K. pneumoniae* (then known as *Aerobacter aerogenes*) required 25 to 30 times more histidine for growth when inositol was provided as a carbon source than when glucose was provided (162). The cause of this increased need for exogenous histidine was that

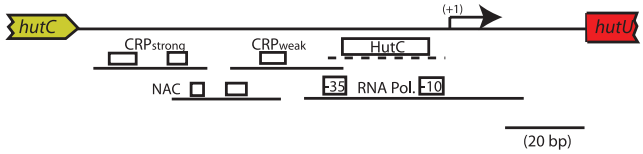

FIG 5 Regulatory sites in the *hutU* promoter region of *K. pneumoniae*. The upper line represents the intergenic region between *hutC* and *hutU*, with +1 indicating the start of transcription of the *hutUHT* operon. Boxes represent matches to consensus sequences for known regulators of *hutUHT* transcription, and the lines beneath the boxes represent the extents of the footprints of those regulators on the DNA. The diagram was drawn approximately to scale.

the histidine was being degraded (86). The degradation of histidine was shown to be inducible in that the Hut enzymes were not present in cells grown with glucose or glutamate in the absence of histidine (90), and the rate of histidine degradation was correlated with the quality of the carbon source provided, with glucose being the most repressing carbon source (86, 90). Moreover, the induction by histidine and the repression by glucose were independent (91). The repression of histidase by glucose was overcome if ammonium was omitted from the growth medium (105). Thus, three of the regulatory mechanisms that affect the Hut system of *K. pneumoniae*, i.e., operon-specific induction, carbon catabolite repression, and nitrogen regulation, were identified early. The mechanisms that govern these three regulatory phenomena are well understood. The proteins responsible for these regulatory effects, i.e., HutC, CRP, and the nitrogen assimilation control protein (NAC), are described below, and their binding sites within the very crowded *hutU* promoter region are illustrated in Fig. 5. A fourth mechanism, repression under anaerobic conditions, was identified in a related organism, *Klebsiella oxytoca* strain M5aL (44). This repression has also been noted for *K. pneumoniae*, but the effect is considerably weaker (unpublished observation). The mechanism of this anaerobic repression is unknown.

Induction of Hut. The true physiological inducer of Hut in *K. pneumoniae* is urocanate. The choice of an intermediate as inducer guarantees that *hut* will not be induced unless histidine is present, abundant in the environment, and continuously available. If histidine is present at low concentrations, its transport will be balanced by its incorporation into proteins, and intracellular levels will not rise high enough to allow enough urocanate accumulation to cause induction of *hut*. The K_m for histidine is not known for the histidase from enteric bacteria but is probably similar to that measured for the histidases of *B. subtilis* and *P. fluorescens* (3.9 mM and 2.8 mM, respectively) (51, 132). The K_m of the tRNA synthetase from *S. enterica* is considerably lower, variously measured as 150 μM or 87 μM (36, 131). That for the *E. coli* enzyme is even lower (36), and that for the *K. pneumoniae* enzyme is probably also quite low. Thus, it seems probable that the affinity of histidyl-tRNA synthetase for histidine exceeds that of histidase by a factor of at least 20. As a result, constitutive expression of *hut* does not lead to a histidine requirement for growth. Nor does it lead to an increase in the levels of the histidine biosynthetic enzymes (137), whose transcription would be increased if the pool of histidyl-tRNA were uncharged with histidine. Thus, the intracellular pool of endogenously produced histidine is insufficient to cause urocanate-mediated induction unless urocanase is inactivated.

K. pneumoniae has a specific urocanate permease and thus can

grow with urocanate as the sole source of carbon or nitrogen. Since urocanate is the direct inducer of *hut*, it might seem that small amounts of urocanate might lead to premature induction of *hut* in this organism. However, here too there is a regulatory circuit that guarantees induction only when urocanate is present, abundant in the environment, and continuously available. The urocanate transport gene *hutT* (as part of the *hutUHT* operon) is itself inducible by urocanate. As shown many years ago by Cohn and Horibata for the *lac* system (18, 19), the presence of low levels of inducer or brief exposures to inducer can be balanced by degradation of the inducer to prevent full induction. Only when enough inducer accumulates does the system switch to a fully induced state. Since urocanase and the urocanate transporter are coexpressed, the induction mechanism is desensitized to small amounts of urocanate.

Induction of Hut is controlled by a repressor, HutC, the product of the *hutC* gene. Mutations that constitutively express Hut were mapped to *hutC* (15, 45, 100, 150) and were found to be recessive to the wild type in both *K. pneumoniae* (45) and *S. enterica* (150). Rare "superrepressor" mutants of *S. enterica* with mutations in *hutC* were uninducible by urocanate or imidazole propionate (48). These mutations were dominant to the wild type and readily reverted to a Hut-constitutive (inducer-independent) phenotype (48). Taken together, these data strongly suggested that HutC is a repressor. DNA-binding studies with purified HutC from *S. enterica* showed that HutC bound to the operator regions of both the *hutUHT* and *hutIGC* operons and that binding to either of the operator regions was abolished if urocanate or imidazole propionate was present (49). The DNA sequence of the HutC protein from *K. pneumoniae* (139) and its DNA-binding site (118) strongly resemble the corresponding elements from *P. putida* (2), demonstrating that the mechanism of induction is conserved (see below).

The induction of Hut by histidine requires an active histidase to generate urocanate and a balance between histidase and urocanase activities to allow accumulation of urocanate, which may explain the cotranscription of *hutU* and *hutH*. Mutants lacking histidase activity are inducible by urocanate but not by histidine (137). Mutants lacking urocanase express Hut at constitutively high levels in the absence of any inducer (137). Recall that mutants expressing *hutUH* but not *hutIG* are poisoned by histidine (14, 48), so it is important that induction of the two operons be coordinated both in time and in degree. In *S. enterica*, this is achieved by having *hutC* be part of the *hutIGC* operon and by the fact that the *hutIGC* operator region has a lower affinity for HutC than does the *hutUH* operator (49). As a result, the *hutUH* operon is more repressible than the *hutIGC* operon (148), and *hutUH* expression will always be fully repressed before HutC can fully repress its own expression from *hutIGC*. In *K. pneumoniae*, the *hutIGC* operon is strongly repressed by HutC (45), so a different strategy is needed to prevent repressor levels from falling too low to keep *hutUHT* expression below the level of *hutIGC* expression. Thus, in *K. pneumoniae*, *hutC* can be expressed from a separate promoter (142). Binding studies with the *K. pneumoniae* operator regions are lacking.

The identification of imidazole propionate, a urocanate analog that can induce *hut* but cannot be metabolized by *K. pneumoniae* (136), simplified studies of induction. Induction by histidine, urocanate, or the nonmetabolizable compound imidazole propionate requires a permease capable of transporting these compounds. The enteric bacteria possess multiple permeases capable of transporting histidine, none of which is induced or repressed by histidine (3). In addition, *K. pneumoniae* expresses a Hut-specific, inducible permease (HutT) that can transport either urocanate or imidazole propionate, but probably not histidine (136). In contrast, *S. enterica* lacks a HutT homologue, and thus imidazole propionate can induce *S. enterica hut* expression only at very high concentrations and urocanate cannot induce *hut* expression at all (15, 100).

Carbon catabolite repression. In *K. pneumoniae*, histidine is not degraded if glucose is provided (86). The same is true for *S. enterica* (15, 100). Furthermore, the effect is not specific to glucose. Histidine degradation is repressed by any carbon source that allows faster growth than histidine (86). This observation formed the basis for the concept of catabolite repression (87), whereby the expression of genes for the utilization of poorer carbon sources is limited by the presence of better carbon sources. The observation that growth in the absence of glucose leads to an increase in cyclic AMP (cAMP) led to the suggestion that cAMP might be a signal for this effect (93). Decades of work in dozens of laboratories have confirmed a role for cAMP and its intracellular receptor protein, CRP (also known as CAP), in the regulation of many catabolic operons, including *hut*. Addition of cAMP to the growth medium overcomes glucose-mediated catabolite repression of histidase formation in *K. pneumoniae* (124). Mutants defective in the genes for adenylate cyclase (*cya*) or CRP (*crp*) cannot activate *hut* expression in response to carbon limitation (115, 124). *In vitro* transcription with purified components showed that both CRP and cAMP were necessary (and sufficient) to activate transcription from *hutUp*, the *hutU* promoter (115, 116). The *hutUp* region contains two CRP-cAMP-binding sites: a stronger site centered at position −82.5 (relative to the start of transcription) and a weaker site centered at position −42.5 (116, 117). The stronger site is essential for activation of *hutUp* by CRP-cAMP (117). The weaker site also appears to play a role in activation, but this is not well characterized (117).

The complex connection between glucose and cAMP has been studied extensively in *E. coli* (23). In its simplest form, the model states that the phosphorylated form of the glucose transport protein $EIIA^{gluc}$ (the product of the *crr* gene) activates adenylate cyclase to produce cAMP. Transport of glucose across the cell membrane (with its concomitant phosphorylation) results in dephosphorylation of $EIIA^{gluc}$. This in turn leads to a loss of adenylate cyclase activity, a reduction in intracellular cAMP, and a failure to activate transcription. Moreover, when the transport of glucose or other sugars is in excess of the cell's biosynthetic needs (e.g., when nitrogen is limiting), α-ketoglutarate accumulates and directly inhibits enzyme I of the phosphotransferase system (PTS), preventing phosphorylation of $EIIA^{gluc}$ as well as other PTS proteins (24) and leading to a loss of adenylate cyclase activity.

The connection between glucose and catabolite repression appears to be somewhat more complex in *K. pneumoniae* than in *E. coli*. First of all, repression of plasmid-borne *hut* by glucose is significantly more severe in a *K. pneumoniae* cell than in an *E. coli* cell, and this is true whether the plasmid-borne *hut* gene is from *K. pneumoniae* or *S. enterica* (43). This suggests that glucose is more effective at reducing cAMP levels or that other mechanisms of catabolite repression are effective under these conditions. A second difference between *K. pneumoniae* and *E. coli* is that mutants of *K. pneumoniae* that cannot phosphorylate $EIIA^{gluc}$ (*ptsH* or

ptsI) still express *hut* (and *lac*) at high levels, even in the presence of glucose, as do *crr* mutants that lack EIIA^{gluc} (5). These observations may reflect differences in glucose metabolism between these organisms. As glucose becomes less limiting (more abundant), *K. pneumoniae* reduces the amount of glucose carried by the PTS transport system (106), and as much as 75% of the glucose used is consumed by a periplasmic glucose dehydrogenase (107). In other words, under catabolite repression conditions (glucose excess), much of the glucose metabolism is via glucose dehydrogenase. Consistent with this, repression of *hut* (and *lac*) expression by glucose (but not other sugars) is abolished in *K. pneumoniae* mutants that lack glucose dehydrogenase (104). Glucose dehydrogenase requires an unusual quinone cofactor, pyrroloquinoline quinone (PQQ), for its activity (25). Although *E. coli* has the gene for glucose dehydrogenase and synthesizes the apoenzyme in a regulated way (39), *E. coli* lacks the genes or capability for PQQ synthesis and cannot form an active glucose dehydrogenase in pure culture unless PQQ is provided in the medium (98). However, *E. coli* is chemotactic toward PQQ when glucose is present (22) and thus can probably activate glucose dehydrogenase in mixed cultures. This PQQ-dependent glucose dehydrogenase donates electrons to the electron transport chain, allowing the generation of a proton motive force without the need for transport of glucose across the cell membrane or the generation of potentially toxic sugar phosphates (50). As a result, cells are able to balance the energy yield from glucose and the need for carbon skeletons for biosynthesis (159, 160). In any event, even though it was the study of *hut* expression in *K. pneumoniae* that first led to the definition of the term "catabolite repression," it is clear that there are still elements of this phenomenon that bear investigation.

Nitrogen regulation. The repression of *K. pneumoniae hut* expression by glucose can be overcome if the cells are grown under nitrogen-limiting conditions (105, 124). The study of this phenomenon was delayed until a practical system of genetic analysis became available for *K. pneumoniae* (42, 85). A role for the biosynthetic enzyme glutamine synthetase (GS) was demonstrated in that mutants lacking GS are unable to activate *hut* expression in the presence of glucose and mutants that form GS constitutively express *hut* constitutively as well (123). This reflects the fact that the size of the intracellular pool of glutamine is a key surrogate for the nitrogen of the enteric bacteria during steady-state growth (9, 61).

The activation of *hut* transcription in response to nitrogen limitation is achieved by NAC, which was reviewed recently (8). In brief, NAC is a LysR-type transcriptional regulator (140) which activates transcription of *hutUH* by an RNA polymerase that carries σ^{70} as its sigma factor (46). Mutants that lack NAC cannot activate *hut* expression in response to nitrogen limitation, although the response of *hut* to carbon limitation remains intact (10). Mutants that express NAC constitutively have high levels of *hut* expression even under conditions of nitrogen excess (141). Thus, NAC is both necessary and sufficient for activation of *hut* expression. The effect of NAC is specific for activation of a subset of nitrogen-regulated operons that includes *hutUH* and about 100 other operons (37) but not all nitrogen-regulated genes (7, 84). NAC activates *hutUH* transcription by binding to a site centered at position −64 relative to the start of transcription (46). The details of this site are poorly understood, but binding of NAC to this site results in a conformational change that is necessary for activation of transcription (120). It is assumed that NAC also activates *hutIG* expression, because at least *hutG* responds to nitrogen limitation (91). Furthermore, if *hutUH* expression is activated by inducing *nac*, no histidine toxicity is observed as would be expected if *hutUH* expression outstripped *hutIG* expression (14, 48). However, the role of NAC in activation of *hutIG* expression has not been confirmed.

NAC is unusual among regulators in that no coeffector is involved in regulating its activity and all regulation by NAC is controlled at the level of transcription of the *nac* gene (46, 141). The expression of *nac* (and thus of *hut*) in response to nitrogen limitation is ultimately controlled by the Ntr system, which has been reviewed elsewhere (88, 126).

In brief, the Ntr system is a global regulator based on two proteins: NtrB and NtrC. NtrC is a DNA-binding protein which, when phosphorylated (NtrC∼P), can activate transcription by an RNA polymerase bearing the unusual sigma factor σ^{54} (56). NtrB is a complex protein that phosphorylates NtrC or dephosphorylates NtrC∼P (69, 111) in response to signals from a regulatory protein, PII.

The activities of PII are themselves regulated by covalent modification (uridylylation) (92). The uridylylation state of PII reflects the intracellular pool of glutamine (66). It also reflects the intracellular pool of α-ketoglutarate, which both modulates the effect of glutamine (110) and affects the physiological balance between carbon and nitrogen (24), as well as the adenylate charge of the cell (65). This allows the cell to monitor the nitrogen supply both independently and in the context of its carbon and energy supply (144, 171). Thus, when glutamine pools are low, the kinase activity of NtrB is dominant and NtrC∼P accumulates, allowing expression of Ntr-dependent genes. When glutamine pools are high, the phosphatase activity of NtrB is enhanced and NtrC∼P is inactivated to NtrC.

Under high-glutamine (nitrogen excess) conditions, there is very little NtrB or NtrC in the cell, and a shift to nitrogen-limiting conditions requires a separate mechanism to "jump-start" the system. When nitrogen is suddenly limiting, carbon metabolism outstrips biosynthetic activity, and α-ketoglutarate (171) and acetyl phosphate (75) can accumulate. α-Ketoglutarate slows glucose catabolism, allowing nitrogen metabolites to "catch up" (24), and acetyl phosphate phosphorylates NtrC to NtrC∼P nonenzymatically (30). Cells that cannot accumulate acetyl phosphate have difficulty making the transition from nitrogen excess to nitrogen limitation. Thus, during a shift from nitrogen excess, the cells first sense the limitation by the accumulation of acetyl phosphate. This then leads to an autoregulatory loop whereby NtrC∼P activates expression of the genes for NtrC and NtrB, greatly increasing their intracellular concentration. The NtrB response to the low glutamine pools (resulting from nitrogen limitation) then maintains the high levels of NtrC∼P that are required for *nac* expression both *in vivo* (84) and *in vitro* (31).

To summarize, the available evidence is consistent with the following picture. Nitrogen limitation is sensed either as accumulation of acetyl phosphate and α-ketoglutarate (excess of carbon and energy metabolism over biosynthetic capacity) or by low glutamine pools. This leads to activation of NtrC to NtrC∼P. NtrC∼P activates *nac* gene expression, and finally, NAC activates transcription of *hut*. The three commonly studied enteric bacteria differ from each other with respect to nitrogen regulation of *hut* expression. *S. enterica* lacks a *nac* gene (11, 47), but its *hut* operons still retain all the signals needed to respond to the NAC from *K.*

pneumoniae or *E. coli* (11, 43, 46). *E. coli* lacks the *hut* operons, but its NAC protein is able to activate the *hut* operons from *K. pneumoniae* or *S. enterica* (43, 46, 103).

Anaerobic repression. Growth of a *K. oxytoca* strain under anaerobic (fermentation) conditions leads to a strong repression of *hut* expression that is not overcome by nitrogen or carbon limitation (44). This effect is not the result of a failure of nitrogen regulation or carbon regulation, since glutamine synthetase and urease are still activated by nitrogen limitation and β-galactosidase is still activated by carbon limitation under anaerobic conditions (44). A similar, though much less significant, repression is seen in *K. pneumoniae* as well (unpublished observation). The mechanism of this regulatory effect is unknown. The physiological significance of the effect may reflect the fact that utilization of histidine as a carbon source ultimately implies that glutamate is the carbon source. Enteric bacteria would be able to use glutamate as a carbon and energy source only under respiratory, not fermentative, conditions. Unfortunately, it is not known whether *hut* expression requires oxygen or merely an electron acceptor. The effect was not tested under conditions of anaerobic respiration (e.g., with nitrate as an electron acceptor).

Pseudomonads

The *hut* operons of the pseudomonads are also subject to induction, carbon catabolite repression, and nitrogen regulation, though the last two are achieved by very different mechanisms in the pseudomonads than in the enteric bacteria. The proteins responsible for these regulatory effects (HutC, CbrB, and NtrC, respectively) are discussed below.

Induction of Hut. The ability of *P. fluorescens* to degrade histidine has been known to be an "adaptive" (i.e., inducible) property since the early 1950s (153). The same was shown to be true for both *P. aeruginosa* and *P. putida* (80, 81). Like the case with the enteric bacteria, the physiological inducer of *hut* expression in the pseudomonads is urocanate, the first intermediate in the pathway (80, 81, 175). The HutC (repressor) protein from the pseudomonads is highly similar to that from the enteric bacteria, with 62% identity between the *P. putida* and *K. pneumoniae* HutC amino acid sequences (2, 139). Despite the similarity in structure, there are some differences in the function of the HutC protein from pseudomonads. In the absence of urocanate, HutC binds to three sites in the *hut* cluster, including one in front of the *hutUHIG* operon of *P. putida* or the equivalent operons of other pseudomonads, one in the region between the divergently transcribed *hutCD* and *hutF* operons, and one in a region preceding the *hutG* gene (2, 175). The *hutF* and *hutUHIG* operons appear to be inducible by urocanate alone, but HutC-mediated repression of *hutG* expression is relieved by either urocanate or formylglutamate (2, 57). Formylglutamate does not induce either *hutUHIG* or *hutF*; however, it does not inhibit their induction by urocanate, suggesting that HutC from the pseudomonads has a binding site for formylglutamate that is separate from the urocanate-binding site (2). The HutC-binding site that regulates the *hutU-G* operon of *P. putida*, *P. fluorescens*, and *P. aeruginosa* is highly conserved, both in sequence and in position (overlapping the RNA polymerase-binding site of the promoter). This allows a convenient alignment of the regulatory sites in this complex promoter region.

The *hutC* gene in pseudomonads is followed immediately by *hutD*, whose function is not well understood. It is not required for repression by HutC or induction by urocanate *in vivo* or *in vitro* (2) but appears to act as a governor that may limit overexpression of *hut* under some conditions (174). Although the role of HutC in the repression of *hutF*, *hutHUIG*, and *hutG* is clear, it is not known whether it also represses *hutCD* expression. The presence of the HutC-binding site in the space between the divergently transcribed *hutF* and *hutCD* operons could function to repress both operons, and the analogy to the case in the enteric bacteria makes such a speculation attractive, but there is no direct demonstration of such autoregulation of HutC production (2).

HutC from pseudomonads is also unusual in that it represses not only σ^{70}-dependent transcription but also the σ^{54}-dependent transcription from the *hutU* promoter (175). The mechanism of this repression will likely be interesting, since σ^{54}-dependent transcription is generally not subject to repression.

Carbon catabolite repression. Hut expression in the pseudomonads, like that in enteric bacteria, is repressed when a better carbon source is provided, and this repression is relieved in the absence of the preferred nitrogen source, ammonium (81). However, this phenotypic similarity is deceiving. The mechanisms of carbon catabolite repression in the pseudomonads are not yet fully understood, but they are clearly very different from those of the enteric bacteria, as documented in recent reviews (20, 62, 130). Most of the key features of the enteric mechanism for catabolite repression are absent from the pseudomonad mechanism. For example, the central role of glucose is not seen in *Pseudomonas*, where succinate and other tricarboxylic acd (TCA) cycle intermediates are the preferred carbon and energy sources (81). Most importantly, cAMP and the PTS play no role in catabolite repression in *Pseudomonas* (119). Finally, there appear to be several, possibly independent mechanisms that operate to create a balance between carbon and energy metabolism and biosynthetic requirements (130). What is clear is that the two-component CbrAB system is important for activation of the *hut* genes from *P. fluorescens* and *P. aeruginosa* in response to carbon limitation (176). Mutants lacking CbrA or CbrB cannot grow with histidine (or several other amino acids) as the sole carbon source, but they grow normally with histidine as the sole nitrogen source when glucose or succinate is present as a carbon source (112, 176). When histidine is the sole carbon source, the *hutU-G* operon of *P. fluorescens* is transcribed from a σ^{54}-dependent promoter and the *hutF* gene is transcribed from a σ^{70}-dependent promoter (176). This suggests that CbrB may directly activate σ^{54}-dependent transcription of *hutU-G*, but this has not been demonstrated. It remains an open question whether CbrB activates *hutF* transcription directly or at all. Unpublished results cited by Itoh et al. (62) suggest that a CbrB-binding consensus sequence is located about 260 bp upstream from the HutU coding sequence of *P. aeruginosa*. Both the sequence (TGTTACCGAA) and position of this predicted CbrB-binding site are conserved between *P. fluorescens* and *P. aeruginosa*, as would be expected from their close phylogenetic relationship, supporting the importance of this site. Although *P. putida* also appears to be subject to carbon catabolite repression (21, 60), no match to this sequence is immediately apparent in the *hutU* promoter region of *P. putida*.

Nitrogen regulation. When histidine serves as the sole nitrogen source for *P. fluorescens*, both the *hutU-G* and *hutF* operons are transcribed from σ^{70}-dependent promoters. Regulation of this transcription is complex. When histidine is the sole nitrogen source, cells lacking CbrB have about 3-fold lower expression of *hutU-G* than wild-type cells but grow well on succinate (or glu-

cose) plus histidine. This residual expression of *hutU-G* is entirely dependent on another two-component regulator, NtrBC. In the absence of both CbrB and NtrC, *hutU-G* expression is not activated and the cells cannot grow with histidine as the sole carbon source or sole nitrogen source (176). In other words, cells with an active CbrAB system can use histidine as the sole carbon or nitrogen source and do not require NtrBC under either condition. However, transcription of *hutU-G* shifts from a σ^{54}-dependent promoter when histidine is the sole carbon source to a σ^{70}-dependent promoter when histidine is the sole nitrogen source (176). When the CbrAB system is inactive, the NtrBC system can provide a smaller (but still sufficient) amount of *hutU-G* transcription from the σ^{70}-dependent promoter to allow growth with histidine as the sole nitrogen source (176).

The regulation of *hut* expression in *P. fluorescens* raises several important questions. Is the regulation of *hutU-G* by either CbrB or NtrC direct, or does one or the other system (or both) require an intermediate analogous to the NAC protein of the enteric bacteria? It is clear that the *P. fluorescens* protein with the greatest sequence similarity to the *K. pneumoniae* NAC protein is not involved in nitrogen metabolism in *P. fluorescens* (176). But the fact remains that CbrB is required for both σ^{54}-dependent and σ^{70}-dependent transcription of *hutU-G*, and NtrC, which is generally thought to activate σ^{54}-dependent promoters, is required for σ^{70}-dependent expression of *hutU-G*. Regulation of the *hutF* transcript remains relatively poorly understood, and there is virtually no information about the *hutCD* transcript. In the absence of *in vitro* studies with purified components, these questions remain open.

Expression of the *hutU* and *hutH* genes of *P. aeruginosa* is also activated under conditions of nitrogen limitation (122). Unpublished observations cited by Itoh et al. (62) claim to identify two NtrC-binding sites in *P. aeruginosa*, located about 240 and 270 bp upstream from the HutU coding sequence. These sites show considerable similarity to the consensus binding site for NtrC from *E. coli* (88). A similar match to the *E. coli* consensus is found in *P. fluorescens*, in the same relative location (i.e., adjacent to and downstream from the predicted CbrB-binding consensus), suggesting that the regulation may be similar in both species. *P. putida* is also subject to regulation by carbon and nitrogen (21), but the regulatory region preceding the *P. putida hutU-G* operon is shorter than that in *P. fluorescens*. No site resembling the CbrB-binding site has yet been identified in this region, but a sequence with similarity to the NtrC-binding site is located within the *hutD* gene, placing the site about the same distance from *hutUp* as in the other two species. Clearly, there are many important questions remaining regarding the carbon and nitrogen regulation of the pseudomonads, including whether the regulation by CbrB and NtrC is direct or indirect and whether the predicted binding sites function in regulation. Resolution of these questions awaits studies of *in vitro* transcription with purified components.

Temperature regulation. About 5% of the genes expressed at 20°C by an Antarctic psychrotrophic *Pseudomonas syringae* strain are expressed at significantly higher levels at 4°C (68). The histidase and urocanase genes are among them, with urocanase activity being 14-fold higher in cells grown at 4°C than at 20°C, in marked contrast to the result seen with a mesophilic pseudomonad, where a lower temperature resulted in lower urocanase activity (68). The mechanism responsible for this increased activity at low temperatures is unknown, but it operates at the transcriptional level and may reflect a cold-active promoter upstream from the normal promoter that operates at both temperatures (64). The physiological significance of this regulatory effect is also unknown.

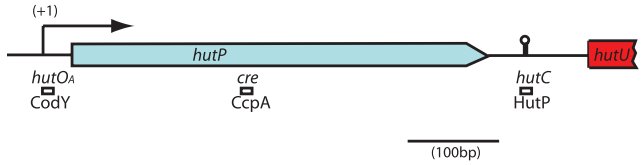

FIG 6 Regulatory sites in the control region of the *hut* operon of *B. subtilis*. The upper line represents the control region of the *hutHUIGM* operon, including the start of transcription (+1) and the *hutP* gene. Boxes below that line indicate the binding sites for the following regulatory proteins: CodY, a transcriptional repressor that binds to the *hutO$_A$* site; CcpA, a protein that blocks transcription by binding at a *cre* site; and HutP, which prevents transcriptional termination at the *hutC* site, indicated by the lollipop structure on the upper line. The diagram was drawn approximately to scale.

Bacillus subtilis

The regulation of Hut expression in *B. subtilis* is very different both physiologically and mechanistically. The sites where regulatory proteins act to regulate *hut* operon expression in *B. subtilis* are illustrated in Fig. 6.

Induction of Hut. The Hut enzymes of *B. subtilis* are induced by growth in the presence of histidine and are absent when histidine is omitted from the growth medium (51). However, in contrast to the situation with the enterics and the pseudomonads, the physiological inducer for *B. subtilis* Hut expression is histidine, not urocanate (17). Moreover, the mechanism of induction in *B. subtilis* is entirely different from the repressor-mediated control seen in the enterics and the pseudomonads. Mutations (*hutC*) that allow *hut* expression in the absence of inducer were difficult to obtain, but mutations (*hutP*) that greatly reduce expression of all *hut* products were readily obtained. Moreover, the vast majority of *hutP* revertants had a wild-type (inducible) phenotype (17). The *hutP1* mutation, which renders *hut* noninducible (pleiotropically negative), was cloned and sequenced and shown to be recessive to the wild-type sequence (114). This is the pattern one would expect for a positive regulator rather than for a repressor. In fact, the inducibility of *hut* is accomplished principally by a termination-antitermination mechanism (165). Transcription of the wild-type *hutPHUIG* operon begins just upstream of the *hutP* gene (114) and terminates at a stem-loop structure between *hutP* and *hutH*, both *in vivo* and *in vitro* (165). When a *hutC* mutant was analyzed, transcription did not terminate at this site, and sequence analysis of the *hutC1* mutation showed that the mutation destabilized the stem-loop structure, weakening its antitermination effectiveness (165). Although this antitermination mechanism accounted for most of the difference between induced and uninduced levels of Hut expression, there was also a 5-fold increase in transcription initiation from the promoter of the *hutPHUIG* operon in the presence of histidine (113, 165). Thus, the data show that HutP activates the promoter of the *hut* operon of *B. subtilis* about 5-fold and that HutP also allows readthrough of a powerful termination signal located between *hutP* and the enzyme-encoding genes of the operon.

X-ray crystallography has revealed the structural basis of HutP-mediated antitermination (79). In brief, a hexamer of HutP binds histidine and undergoes a conformational change. It then

binds a magnesium ion and undergoes another conformational change. The HutP-histidine-Mg^{2+} complex then binds two regions of the GC-rich stem-loop (terminator) of *hutC*, destabilizing the structure and allowing readthrough (79). The mechanism by which HutP activates transcription from the *hutP* promoter remains unknown.

The fact that the genes for both the transporter and the degrader of the inducer (*hutH* and *hutM*) are coexpressed guarantees that induction of Hut in *B. subtilis* will not occur unless histidine is present, abundant in the environment, and continuously available. When histidine is present at low levels, residual (uninduced) expression of histidase can degrade it before much induction can occur. As levels of the transporter rise and the amount of exogenous histidine remains high, histidine can accumulate to levels where induction becomes inevitable. Under these conditions, the inducer-degrading enzyme is present at high levels. Thus, when the exogenous histidine is exhausted, induction of the operon is quickly reversed. In other words, the regulatory circuitry used by *B. subtilis* to prevent premature induction of Hut by histidine is the same as that used by *K. pneumoniae* to prevent premature induction of Hut by urocanate (but different from that for histidine).

Carbon catabolite repression of Hut. Two different global regulators operate to control the flow of carbon in the metabolism of *B. subtilis* (145). One of these, CodY (147), responds to both amino acid availability and general nutritional status, and the other, CcpA (53), responds to the quality of the carbon source. Despite the obvious overlapping roles of these two regulators in general metabolism (145), their roles in the regulation of the *B. subtilis hut* genes are quite distinct, and the amino acid repression of *hut* is treated separately here. In contrast to the *hut* operons of the Gram-negative bacteria, the *hut* operon of *B. subtilis* is not derepressed in response to nitrogen limitation (17, 138) and is not under the direct control of the *B. subtilis* nitrogen regulatory systems (34).

The *hut* operon of *B. subtilis* is strongly repressed by glucose and other carbon sources, with the degree of repression reflecting the quality of the carbon source (17). *cis*-acting mutations that render *hut* resistant to repression by glucose were identified and shown to lie near mutations that defined the promoter and *hutP* (17). In fact, these mutations lie within the *hutP* coding sequence, about 215 nucleotides downstream from the start of transcription (113, 166). These mutations lie in a region with strong similarity to *cre* sites, which have been implicated in catabolite repression of several other operons in Gram-positive bacteria (59). A second site ($hutO_A$), responsible for a weak (about 2-fold) repression by glucose, was initially reported about 10 to 20 bp downstream from the operon's promoter (166), though its role in carbon catabolite repression is disputed (26). The role of this promoter-proximal site is complicated because the same region appears to be involved in the CodY-mediated amino acid repression described below (26, 166). The CcpA protein has been shown to be required for carbon catabolite repression of *hut* in *B. subtilis* (166). Since the sequence of the *cre* site within *hutP* strongly resembles *cre* sites that are known to bind CcpA (73), it seems certain that binding of CcpA to the *cre* site is responsible for the carbon catabolite repression of *hut* expression in *B. subtilis*. The physiological link that connects the quality of the carbon source to the binding of CcpA at *cre* sites remains elusive. There are reports that phosphorylated compounds such as glucose-6-phosphate and fructose-1,6-bisphosphate (101) or the phosphorylated form of the HPr protein (38) enhance binding of CcpA to *cre* sites in other *B. subtilis* operons. Binding of CcpA at the *hut cre* site has not been demonstrated directly, but it seems very likely. In fact, mutations in the *mfd* gene (which encodes a transcription repair coupling factor) partially relieve catabolite repression of *hut*, suggesting that CcpA (or something controlled by it) forms a transcriptional block at the *cre* site (172).

Amino acid repression. Although *B. subtilis* has a complex nitrogen regulatory system that affects many nitrogen-yielding operons (34), the *hut* operons are not regulated in response to nitrogen limitation in this organism (17, 138). However, *hut* transcription is strongly repressed when cells are grown in the presence of a mixture of 16 different amino acids (4). A mixture of 11 amino acids was as repressing as the full 16, at least in the presence of glucose, but mixtures of 3, 5, or 6 amino acids were only partially repressing (4). This amino acid-mediated repression acts by two different mechanisms: inhibiting histidine transport (thus blocking induction) and repressing transcription of *hut* (165). Little is known about the former mechanism. Repression of transcription requires both an operator site ($hutO_A$), located just downstream from the *hut* promoter (165), and a *trans*-acting regulatory protein, CodY (35). The *codY* gene was originally identified as being involved in the control of the *dpp* operon (147). CodY is one of the three global regulators in *B. subtilis* (151), along with CcpA, which regulates *hut* expression in response to carbon availability, and the nitrogen regulator TnrA, which plays no role in *hut* regulation. CodY is a DNA-binding protein whose activity reflects the general nutritional state of the cell and regulates a wide variety of operons besides *hut*. The DNA-binding activity of CodY is stimulated by binding to GTP and branched-chain amino acids, in an additive manner, thus responding to multiple physiological situations. Mutants lacking either the $hutO_A$ site or an active CodY protein show no amino acid repression of *hut* and a slightly reduced catabolite repression (35). The role of CodY and $hutO_A$ in catabolite repression has been difficult to demonstrate and may in fact be indirect (26).

In summary, transcription of the *hut* operon in *B. subtilis* is regulated mainly by three proteins acting at three different sites (Fig. 6): CodY, acting at $hutO_A$, just downstream from the promoter, represses transcription in response to amino acid availability; CcpA, acting at the *cre* site in the middle of the *hutP* gene, creates a block to transcription that prevents full expression of HutP as well as that of the rest of the operon; and HutP, acting at a termination signal between *hutP* and the rest of the operon, allows readthrough of the terminator when histidine is present.

THEME AND VARIATIONS ON THE HUT SYSTEM

In describing the three well-characterized Hut systems discussed above, several basic physiological responses are shared, despite the wide divergence in the molecular mechanisms of those responses. Hut is inducible, by urocanate in two cases and by histidine in one case. The *hut* genes are clustered, though the number of operons in the cluster varies from one to four. The expression of the IP-producing enzymes is carefully coordinated with the production of the IP-degrading enzymes to prevent toxic effects of IP. Catabolite repression means that Hut expression is increased when histidine is needed as a carbon or energy source, though by a radically different mechanism in each of the examples described thus far. Hut expression is increased when biosynthesis is limited by ni-

trogenous precursors, either by Ntr-mediated nitrogen regulation or by CodY-mediated amino acid repression. In fact, Hut has been a fertile area of investigation that has uncovered a number of fundamental molecular solutions to physiological challenges in a variety of organisms. Hut has also been studied in a number of organisms other than the ones described thus far. Although those studies are less complete, they demonstrate that even the shared themes just described are not universal. It is instructive to look at a few of these.

Streptomyces spp.

Like the pseudomonads, the streptomycetes use pathway 2 (70, 77). Again, like the case for the pseudomonads, the entire pathway is inducible (70), and the true inducer appears to be urocanate rather than histidine (77). However, Hut expression is not repressed by either glucose or ammonia (70). The identity of the *hutH* gene has been established (167). The *hutH* gene is unlinked to any other *hut* gene and is transcribed as a single-gene operon (168). Although early reports argue that *hutH* transcription is constitutive (78), it now appears that *hutH* is transcriptionally regulated by a repressor, since the presence of high-copy-number clones of the noncoding region upstream from *hutH* causes *hutH* to be derepressed (168). Current annotation suggests that the remaining *hut* genes, though unlinked to *hutH*, may form a single operon elsewhere on the *Streptomyces coelicolor* chromosome (http://www.ecocyc.org/). The regulation of this putative *hut* operon has not been studied beyond the observation that it is inducible. The significance of this genetic separation between histidase and the rest of the pathway is unclear but might suggest that the system is mainly a urocanate-degrading pathway.

The histidase of the streptomycetes is unusual in that it is posttranslationally modified. The nature of the reversible modification is not known, but it appears that under certain conditions, histidase is synthesized in an inactive form. This inactive histidase can be activated by heat treatment, by treatment with a 90- to 100-kDa protein present in wild-type extracts, or (at least partially) by treatment with snake venom phosphodiesterase (78). Inactivation occurs upon induction of sporulation, nutritional downshift, or phosphate limitation (77). Urocanate may also play a role in this regulation, but its role is complicated by the fact that there is disagreement about whether urocanate is necessary for transcription of *hutH* (78, 168). The nature of the factor(s) that activates and inactivates the histidase of *S. griseus* is unknown. Thus, the streptomycetes present two variations from the general themes discussed so far: separation of *hutH* from the rest of the *hut* genes and regulation of the activity of the histidase by posttranslational mechanisms.

Ralstonia eutropha

The *hut* cluster of *R. eutropha* strongly resembles that of the pseudomonads, with two exceptions (http://www.ecocyc.org/). The *hutF* gene is part of a single *hutHUDIFG* operon that includes all of the enzyme-encoding genes. The *hutD* gene (which acts as a governor on Hut expression in pseudomonads) is cotranscribed with the enzyme genes rather than with the regulatory gene. The Hut enzymes of *R. eutropha* are induced by urocanate and weakly repressed by good carbon sources such as succinate or pyruvate (135). Regulation by ammonium appears to be absent, except that ammonium appears to repress the transport of histidine but not urocanate (135).

R. eutropha is a facultative chemolithoautotroph and can use either organic compounds or molecular hydrogen as its energy source (133). Growth of *R. eutropha* with a hydrogen-oxygen gas mixture results in a repression of histidase formation that is even stronger than the catabolite repression elicited by a good carbon/energy source such as pyruvate (134). Curiously, this repression can be relieved by the presence of carbon dioxide, which appears to allow the excess energy generated by hydrogen oxidation to be used by the energy-demanding process of autotrophic carbon fixation (134). Thus, the repressive effect of hydrogen seems to result from an imbalance between energy (or redox) generation and the need for energy and redox in biosynthesis. This is reminiscent of the situation proposed above for catabolite repression in *K. pneumoniae*. In summary, the Hut system of *R. eutropha* is similar to that of the pseudomonads except for the weak catabolite repression, the lack of nitrogen regulation, and the possible regulation of histidine transport. In the heterotrophs, it is not possible to determine whether catabolite repression is imposed by excess carbon or excess energy (though there are hints that energy plays a role, at least in *K. pneumoniae*). In *R. eutropha*, it appears that energy is the key player in catabolite repression, and this provides a fascinating set of questions about the nature of catabolite repression in general.

Caulobacter crescentus

Although growth yield is a strong indicator of which pathway is used for histidine degradation, it is not an infallible indicator. *Caulobacter crescentus* can degrade histidine with the production of formate rather than formamide as an end product (33), consistent with the presence of pathway 2. The *hut* cluster of *C. crescentus* contains orthologs of the pathway 2-specific genes *hutG*(*Ps*) and *hutF*, again arguing for pathway 2. However, growth yields suggest that only two nitrogen atoms are assimilated from histidine, rather than the three expected from pathway 2 (33). This is unexpected, since *C. crescentus* assimilates ammonia efficiently via the glutamine synthetase/glutamate synthase pathway and grows well with glutamate as the sole nitrogen source (28). However, histidine (like many other amino acids) inhibits the growth of *C. crescentus* in minimal medium, at least transiently, and it appears that some metabolic process must occur to detoxify the histidine before growth can occur (32). This sensitivity to amino acids is a fascinating subject in its own right, but it serves here as a caution against overreliance on using growth tests alone to determine which pathway is in use by an organism.

Interactions of Hut with Other Systems

Although it is recognized that the regulation of Hut expression responds to global regulatory signals such as catabolite repression, nitrogen regulation, and amino acid repression, the regulation by the Hut regulators (HutC and HutP) has generally been considered to be specific for Hut expression. But it is becoming increasingly clear that mutations in *hut* genes can have profound effects on systems other than Hut. In considering these mutations, it is important to distinguish whether such effects are direct (e.g., by binding of a regulatory protein or by the action of a specific intermediate in histidine degradation) or indirect (e.g., by a general imbalance of metabolism). Both types of interactions have been observed, and a few are mentioned here as examples.

Sieira et al. have shown that in *Brucella abortus*, the HutC (Hut repressor) protein binds to the promoter of the *virB* operon,

which encodes the type 4 secretion system (146). Binding of HutC to the *virB* promoter results in an increase in *virB* transcription that is not seen *in vitro* or *in vivo* if *hutC* is deleted. It is not clear whether this increase results from a direct activation or from interference with a negative regulator. However, several features of the system have been established (146). The affinity of the *virB* promoter for HutC is 30 times lower than that of the *hut* promoter. HutC expression is induced along with the rest of the *hut* operon. HutC binding to *virB* is abolished at lower levels of urocanate than binding to *hut* (5 μM versus 50 μM). This led to a model whose elements are ripe for an experimental test. In the absence of urocanate, HutC represses its own synthesis to a level too low to activate *virB*, though still high enough to repress *hut*. In the presence of urocanate, HutC formation is induced along with that of the urocanate-degrading Hut enzymes, but *virB* remains unactivated because the urocanate blocks binding of HutC. As the exogenous urocanate is removed, the urocanate-degrading enzymes reduce the intracellular urocanate to a level where HutC again represses *hut* expression, but urocanate degradation proceeds faster than the dilution of HutC protein. Eventually, there is a low enough level of urocanate to allow the (still reasonably high level of) HutC to bind to *virB*. As growth proceeds, HutC is eventually diluted by cell growth to a level that no longer activates *virB*. This would result in a delayed and transient expression of *virB*, which is in fact the pattern of *virB* expression during infection (146). This appears to argue for a direct effect of the Hut system on pathogenicity.

In contrast, Rietsch et al. (129) demonstrated an indirect effect of the Hut system on the type 3 secretion system (T3SS), and thus on cytotoxicity, of *P. aeruginosa*. They found that overexpression of *hutT* (the transporter) led to reductions in the transcription of the T3SS genes, the levels of the T3SS effector molecules (exotoxins), and cytotoxicity and that these reductions required the presence of histidine in the medium and an intact Hut pathway. Whether this reflects a specific effect of Hut or merely relief of nutrient starvation is unknown. A third example of the interaction between Hut and virulence is more than 30 years old. Bowden et al. (13) showed that the histidine-dependent increase in alkaline protease (and decrease in collagenase) in *Vibrio alginolyticus* appears to involve urocanate as the signal. In summary, *hutH* mutants do not show induction of alkaline protease production, and *hutU* mutants show induction and even a partial constitutivity. This resembles the pattern seen for Hut induction in Gram-negative bacteria, as described above. Curiously, however, the induction of Hut in *V. alginolyticus* appears to be mediated by histidine and not by urocanate, in contrast to the Gram-negative paradigm. In wild-type cells, Hut is induced by histidine but not urocanate; in *hutH* mutants, the remaining Hut enzymes are still induced by histidine, arguing that conversion of histidine to urocanate by histidase is required for induction of alkaline protease but not for induction of Hut. This is surprising, given that the *hut* clusters of other *Vibrio* spp. appear quite similar to those of other enteric bacteria. A final example, biofilm formation in *Acinetobacter baumannii*, similarly shows an intriguing role of urocanase in the process. Biofilm formation in *A. baumannii* is much greater when cells are grown with L-histidine as a carbon source than when any other amino acid is used (16). This increase in biofilm formation was blocked by D-histidine and was also much reduced in a mutant lacking the *hutU* gene, perhaps suggesting that one of the later products of histidine degradation (IP or FIG?) might serve as a signal.

These few examples suggest that the seemingly arcane and highly specific pathway of histidine degradation, whose elements are widespread not only throughout the bacteria but also throughout the eukaryotes and, to a lesser extent, the archaea, may have functions beyond merely providing an alternative carbon or nitrogen source.

EVOLUTION AND OTHER OPEN QUESTIONS

The Hut system is a surprisingly widespread and highly conserved pathway. The only difference between the two types of pathway is whether the ammonia present in FIG is "rescued" before FIGase or FGase removes the one-carbon unit. The fact that the genes are almost always clustered strongly suggests that the pathway has spread by horizontal transfer. However, the arrangement of the genes within the cluster is variable, and the regulation of the pathway is so variable as to be almost species specific. In other words, once an organism acquired Hut, it quickly coopted it for its own purposes and under its own regulatory networks, retaining only the enzymatic steps as a constant. The availability of many annotated genomes makes it tempting to attempt to decipher the evolutionary history of the Hut system. A careful evolutionary study is beyond the scope of this review; however, a few comments are worth considering and are probably best left as questions rather than conclusions. It has been suggested that the four-enzyme pathway (pathway 1 in Fig. 1) is primitive and that HutF and HutG(Ps) are adaptations that allowed an extra nitrogen to be extracted, and perhaps an extra carbon as well (58, 62). An extra carbon, in the form of formate, might be especially advantageous for autotrophs with Hut, e.g., *R. eutropha*. Consistent with this evolutionary argument, the HutF enzyme of pathway 2 has a higher affinity for FIG than the HutG enzyme of pathway 1. Thus, if an organism acquired a HutF-HutG(Ps) pair (as in pathway 2), its HutG enzyme (from pathway 1) would become irrelevant and would be lost. Moreover, the poor affinity of HutG for FIG might lead to a larger pool of FIG and the preceding intermediate, IP. Since IP can be toxic, a pathway that could reduce the pool size of IP might be favored. HutFG(Ps) might have evolved from any of a variety of enzymes that deal with acyl glutamates, especially acetyl or succinyl glutamate (58), and HutF could have evolved from any of the many amidases found in the bacterial world. It is curious that many organisms (e.g., *P. fluorescens* and *R. eutropha*) with pathway 2 (yielding formate rather than formamide) have an active formamidase. In contrast, organisms (e.g., *K. pneumoniae* and *B. subtilis*) that have pathway 1 (yielding formamide) do not. An interesting exception is *Bacillus cereus*, where the presence of a formamidase and a four-enzyme pathway suggests that this organism might be able to use all three nitrogen atoms from histidine. However, the Hut system of *B. cereus* has not been characterized yet. Whatever the origins of the divergence in the two pathways, this does not answer the question of where the core pathway came from.

Although the distribution of the two pathways generally correlates with the phylogenetic position of the species, there are exceptions. For example, *Erwinia amylovora*, an enteric bacterium, has the pseudomonad-type pathway (pathway 2 in Fig. 1), including HutF, HutG(Ps), and HutD gene products. *Serratia proteamaculans*, another enteric bacterium, also has the pseudomonad-type Hut system, including HutF, HutG(Ps), and HutD, as well as the

HutH ortholog and the four putative transporter ORFs of unknown function found in many pseudomonads. This naturally raises the question of what the HutH ortholog and the four transporter ORFs do. The HutH ortholog (labeled *orfH* in Fig. 4) is clearly not a histidase. It cannot replace *hutH* (175), and it lacks the Ala-Ser-Gly triad that forms the active site MIO of histidase (Fig. 2). Nevertheless, its presence within the *hut* operons of many organisms suggests that it has something to do with histidine catabolism. The fact that *orfH* is usually accompanied by the four ORFs with possible transport functions seems to argue that they function as a unit to introduce a substrate related to histidine into the Hut pathway. Curiously, *K. pneumoniae* appears to have an ortholog of *hutD* and is linked to orthologs of *orfH* and the four putative amino acid transport genes. Obviously, the roles of these genes and their physiological and evolutionary connection to histidine catabolism remain open questions.

There remains some confusion about nitrogen regulation of Hut in pseudomonads, specifically whether NtrC activates Hut expression to allow histidine to be the sole nitrogen source in mutants lacking CbrB (62, 176). In fact, there remain many questions about the binding of CbrB and NtrC in the *hutU* promoter region and how these proteins function to activate transcription. There may even be questions about which form of RNA polymerase is regulated under these conditions. Clearly, *in vitro* studies with purified components are attractive potential experiments.

One general theme that pervades all of the Hut system studies thus far is the ability to buffer the system such that induction of Hut will not deplete the pool of histidine used for protein synthesis. The affinities of histidase and histidyl-tRNA synthetase for histidine are different, guaranteeing that histidine will not be degraded unless the intracellular pools vastly exceed those required for protein synthesis. Moreover, the *hut* gene products both create and degrade the inducer of the *hut* operons. In the case of Gram-negative organisms, in which urocanate is the inducer, low levels of *hut* expression allow a slow generation of urocanate from exogenous histidine. While the pool of urocanate is small, there is a chance for some induction of *hut* to occur, which allows a more rapid buildup of urocanate and more complete induction. However, if histidine disappears, the urocanase will quickly degrade the larger pool of inducer and reestablish repression of the operon. Even in the case of the Gram-positive organism *B. subtilis*, in which histidine is the inducer, the same pattern can be seen. The induction of *hut* requires a substantial pool of intracellular histidine, and the histidine transporter (HutM) is part of the *hut* operon. Thus, it requires slow accumulation of histidine to induce HutM to allow more rapid accumulation to occur and thus induce Hut expression. Again, when exogenous histidine levels fall, the lack of an effective transporter allows histidine degradation (by HutH) to outpace histidine transport (by the anabolic histidine transporters). Thus, the cell is safeguarded against a situation where Hut would channel histidine into a degradative pathway when it could be used (with enormous energetic savings) for biosynthesis. This has been demonstrated for *S. enterica*, where the presence of multiple copies of active Hut does not result in either a histidine auxotrophy or even bradytrophy (89).

As mentioned in the opening paragraph of this review, the study of Hut regulation has led to the discovery of a number of important regulatory paradigms, including carbon catabolite repression, nitrogen regulation, and autoregulation. However, many of the Hut-related questions that led to these discoveries remain open. The recent discoveries of a key role for α-ketoglutarate (a product of Hut) in glucose transport in *E. coli* and the role of glucose dehydrogenase in the catabolite repression of *K. pneumoniae* by glucose are fertile areas for investigation, especially in light of the success of various metabolic modeling studies. Even more questions remain when one considers the carbon regulation of Hut by CbrB in pseudomonads and by CcpA in *B. subtilis*, and this does not begin to touch on the question of how hydrogen gas regulates Hut expression in *R. eutropha*. Also, in all of these examples, the connection between the preferred carbon source (or energy source) and the actual regulatory protein (CRP, CbrB, or CcpA) is far from completely understood.

The nitrogen regulation of Hut in the pseudomonads and the oxygen regulation of Hut in *K. pneumoniae* are both poorly characterized relative to other systems. In addition, the evolutionary arguments are ripe for a careful study. Until recently, Hut was studied as an isolated unit, with no function other than conversion of histidine to glutamate. But the intermediates in the pathway are quite reactive and may have interesting regulatory effects on other systems beyond those described above for biofilm production or pathogenicity. In short, Hut provides an excellent vehicle to allow probing of regulatory and metabolic issues. It is dispensable, its enzymes are easily measured, its chemistry is filled with novelty, its intermediates are potentially functional as effectors, and there is a long history of unexplained observations dating back more than 6 decades. In a word, the potential of Hut has not yet been exhausted.

ACKNOWLEDGMENTS

I thank Allen T. Phillips for his encouragement and helpful suggestions. I thank Boris Magasanik, who once suggested that Hut might be an exciting area to continue investigating. And I thank all of the members of my laboratory, whose hard work proved him right.

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