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Histidine Biosynthetic Pathway and Genes: Structure, Regulation, and Evolution

PIETRO ALIFANO,[1] RENATO FANI,[2] PIETRO LIÒ,[2] ANTONIO LAZCANO,[3] MARCO BAZZICALUPO,[2] M. STELLA CARLOMAGNO,[1] AND CARMELO B. BRUNI[1]*

Centro di Endocrinologia ed Oncologia Sperimentale del Consiglio Nazionale delle Ricerche, Dipartimento di Biologia e Patologia Cellulare e Molecolare "L. Califano," Università degli Studi di Napoli "Federico II," I-80131 Naples,[1] and Dipartimento di Biologia Animale e Genetica, Università degli Studi di Firenze, I-50125 Firenze,[2] Italy, and Departamento de Biologia, Faculdad de Ciencias, Universidad Nacional Autónoma de México, Mexico 04510 D.F., Mexico[3]

INTRODUCTION

The study of the biosynthetic pathway leading to synthesis of the amino acid histidine in prokaryotes and lower eukaryotes was begun more than 40 years ago (119) and has resulted in the unraveling of many fundamental mechanisms of biology. Together with a few other systems, it can be considered a cornerstone in the foundation and evolving concepts of modern cell biology. For some of us who have been involved with its beauties

* Corresponding author. Mailing address: Dipartimento di Biologia e Patologia Cellulare e Molecolare, Via S. Pansini 5, 80131 Naples, Italy. Phone: 39-81-7462047. Fax: 39-81-7703285. Electronic mail address: brucar@ds.unina.it.

and intricacies for more than 20 years, it is particularly important to remember just a few of the accomplishments that have been obtained and of the scientists who tackled those problems.

The histidine system was of the utmost importance in the definition and refinement of the operon theory. A genetic and biochemical analysis of thousands of mutations in the *his* operon of *Salmonella typhimurium* was performed in the late 1950s and early 1960s in the laboratories of Bruce Ames and Phil Hartman (14, 18, 122, 123, 125). These studies showed that, at variance with the yeast systems (119, 175), the bacterial *his* genes were tightly clustered. Demonstration of coordinate expression of this cluster led to the suggestion that this group of genes might function as a single unit of expression and regulation (13). After the formal enunciation of the operon concept (147), Ames, Hartman, and Jacob analyzed 5′-proximal deletions of the regulatory region, resulting in a completely nonfunctional *his* operon, and revertants in which expression of the individual genes was restored to obtain additional evidence of the operon structure (17).

Biochemical studies on the *his* mRNA species synthesized in bacteria were performed by Robert Martin in 1963 (183). Double labeling experiments of constitutive and deletion mutants, RNA chromatographic fractionation, and sucrose gradient centrifugation analysis showed that *his* mRNA is polycistronic and substantiated the one operon–one messenger theory of transcription.

Together with *lac* (207) and *trp* (143), the *his* operon was used as a model system (92, 186) to study the phenomenon of polarity (16, 98, 148). The often hot debate on the mechanisms, translational or transcriptional (188, 196), governing polarity and its implications in general operon function (297) ran into the 1970s (142, 144, 194, 195). Polar mutations in the *his* operon are still used to study fundamental aspects of transcription (6, 8, 64, 65).

Another area in which studies of *his* operon expression were of fundamental help was the study of regulatory mutants and of the mechanisms governing operon expression. Work performed mostly in the laboratories of Ames and Hartman by John Roth identified the different classes of regulatory mutations and showed that, aside from the operator mutations, all caused direct or indirect impairment of the histidyl-tRNA^{His} molecule (20, 91, 235, 236, 253). These findings, in turn, were the basis, together with early studies on the *trp* operon (146), for the identification and elucidation of a novel regulatory mechanism of gene expression, attenuation (38, 294). This term was proposed in 1974 by Takashi Kasai, then working in Phil Hartman's laboratory (156). By performing in vivo and in vitro transcription studies with *his* transducing phages and by measuring *his*-specific RNA in wild-type and operator-constitutive mutants, Kasai identified a transcriptional barrier (the attenuator), the deletion of which in the mutants was responsible for efficient synthesis of downstream mRNA molecules. Although a positive factor was believed to be required in the process, it was also clearly stated by Kasai that the DNA sequence of the attenuator could by itself be responsible for the constitutive expression.

In addition to attenuation, the synthesis of histidine in the cells is also regulated by feedback inhibition (274). Studies of the mechanisms by which the first enzyme in the pathway was inhibited by the end product, histidine, and by some analogs provided important insights in this field of enzymology and regulation of biochemical reactions (18, 184, 250).

These are just some examples of the importance of the histidine system in the evolution of modern concepts of biology. Many more can be found in the classic and lovely book *Gene Action*, written by Hartman and Suskind and published in 1965 in the Prentice-Hall Foundations of Modern Genetics series (126).

Many excellent reviews dealing with several aspects of the histidine pathway have appeared over the years, and readers are referred to them for early aspects (15, 16, 25, 42, 185). In particular, the last comprehensive review on histidine biosynthesis was published in 1987 by Malcom Winkler (289), and a revised version of that review will appear in the second edition of *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology* by the American Society for Microbiology, currently in press (290). In the last 10 years, many studies on the histidine biosynthetic pathway have appeared, dealing with aspects such as gene structure and regulation, transcription initiation and termination, RNA processing, and enzymology. In addition, the system has been extensively investigated not only in enterobacteria but also in many other species (gram-positive and gram-negative bacteria, archaebacteria, and eukaryotic organisms), affording a unique opportunity to study the evolution of this fundamental pathway. In fact, the extensive analysis of the structure and the organization of the *his* genes in the three cell lineages performed in recent years has permitted the recognition of past events of genetic rearrangements, such as gene fusion, gene elongation, and gene duplication. For these reasons, this pathway might be considered a paradigm for the study of the evolution of metabolic pathways. We will try in this review to summarize and describe all these findings in a broader context, as well as to indicate future perspectives and still unanswered questions.

THE HISTIDINE BIOSYNTHETIC PATHWAY

The biosynthesis of histidine has been studied extensively in *S. typhimurium* and *E. coli*. The pathway is described in great detail in the excellent review by Brenner and Ames (42), with particular emphasis on the physiological implications. The pathway is also accurately presented in the review by Malcom Winkler (289). Very recently, important studies which require the modification of previous theories have appeared. In the original studies, the pathway was believed to be composed of 11 enzymatic reactions, since two of the nine genes (*hisD* and *hisB*) encoded bifunctional proteins (16, 126). In later reviews, for unexplained reasons, the dehydrogenase encoded by the *hisD* gene was no longer considered bifunctional, and the steps became 10 (42). The demonstration that *hisI* and *hisE* are, in fact, a single gene (now *hisI*) (61) brought the genes to eight and the steps to 10 (289). Since then, the bifunctional nature of the *hisD* gene product has been reaffirmed (45), but at the same time, it has been discovered that the *hisH* and *hisF* gene products form a heterodimer and both catalyze the same step. The postulated unknown intermediate (42) does not exist (158, 227, 228). In conclusion, three (*hisD*, *hisB*, and *hisI*) of the eight genes of the operon encode bifunctional enzymes, and two (*hisH* and *hisF*) encode polypeptide chains which form an enzyme molecule catalyzing a single step, for a total of 10 enzymatic reactions (Fig. 1).

Reactions and Enzymes in Histidine Biosynthesis

The first reaction in histidine biosynthesis (Fig. 1) is the condensation of ATP and 5-phosphoribosyl 1-pyrophosphate (PRPP) to form *N*′-5′-phosphoribosyl-ATP (PRATP). This enzymatic reaction has been studied in detail by Martin (184) and is the one involved in feedback inhibition. It is catalyzed by the *N*′-5′-phosphoribosyl-ATP transferase, the product of the *hisG* gene. Most of the information about the structure and regulation of the activity of the transferase comes from the

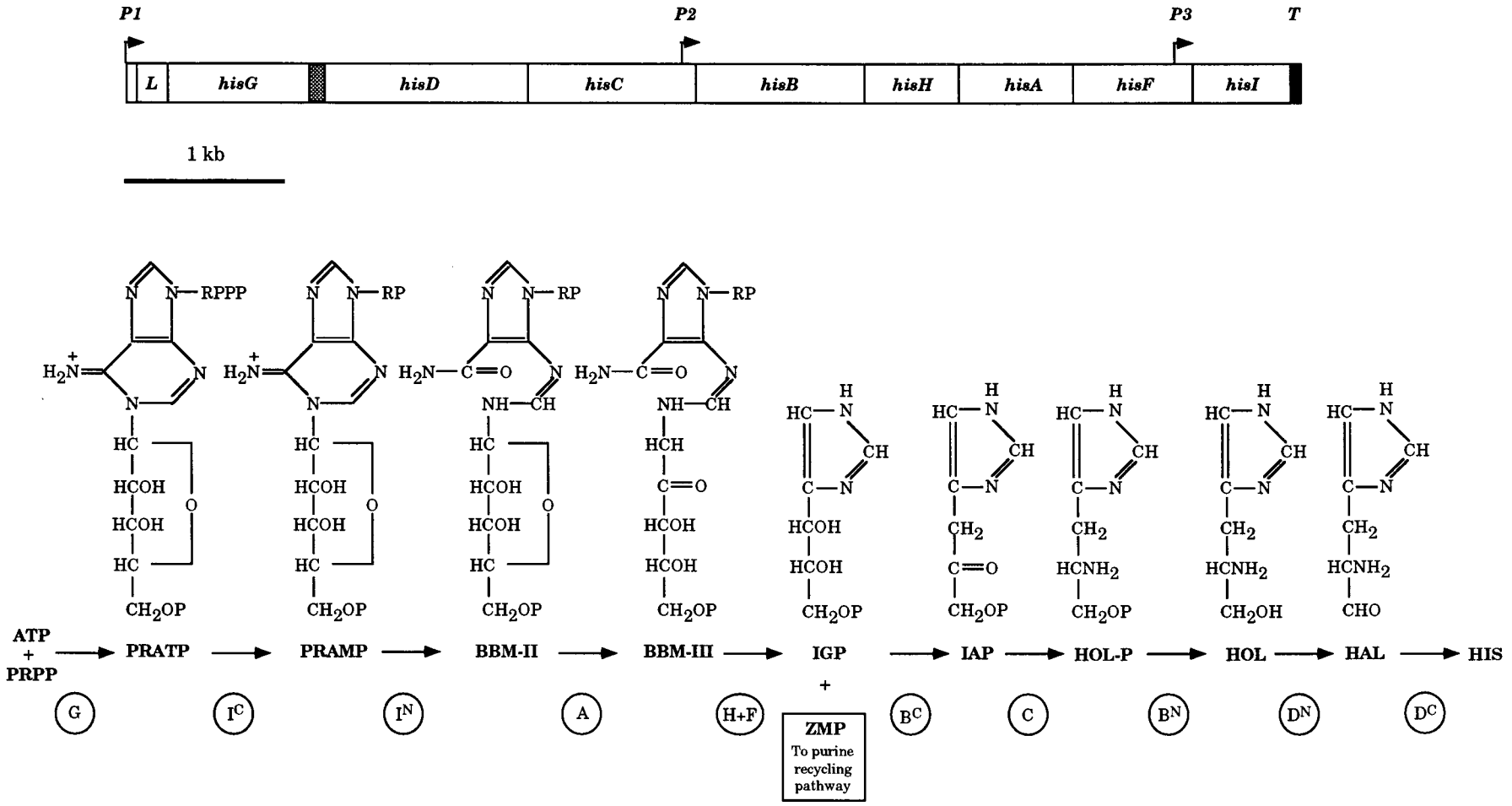


FIG. 1. Gene structure of the *his* operon of *S. typhimurium* and metabolic pathway of histidine biosynthesis. Top: the leader region (*L*) and structural genes are drawn to scale. Arrows indicate the relative positions of *P1* (primary promoter) and *P2* and *P3* (internal promoters). The solid box represents the rho-independent bifunctional transcription terminator (*T*). The shaded box between *hisG* and *hisD* indicates the REP-like element which is absent in *E. coli* and substituted by a 5-bp intercistronic region. Bottom: order of the reactions and the intermediates in the pathway. RPPP, ribosyl triphosphate; RP, ribosyl phosphate; PRPP, 5-phosphoribosyl-α-1-pyrophosphate; PRATP, *N*′-5′-phosphoribosyl-ATP; PRAMP, *N*′-5′-phosphoribosyl-AMP; BBM-II (5′-ProFAR), *N*′-[(5′-phosphoribosyl)-formimino]-5 aminoimidazole-4 carboxamide ribonucleotide; BBM-III (5′-PRFAR), *N*′-[(5′-phosphoribulosyl)-formimino]-5-aminoimidazole-4-carboxamide-ribonucleotide; IGP, imidazole glycerol-phosphate; ZMP (AICAR), 5′-phosphoribosyl-4-carboxamide-5-aminoimidazole; IAP, imidazole acetol-phosphate; HOL-P, L-histidinol-phosphate; HOL, L-histidinol; HAL, L-histidinal (not found as a free intermediate); HIS, L-histidine. The enzymes encoded by the relative cistrons are indicated in the circles below the arrows. When a gene encodes a bifunctional enzyme, the domains performing the single reaction, amino or carboxyl terminal, are indicated by a superscript N or C, respectively.

homologous *S. typhimurium* and *E. coli* enzymes. In both microorganisms, the purified enzyme is a hexamer composed of identical subunits of 34 kDa (161, 215, 278, 288). Multiple aggregation states have been evidenced under different assay conditions. There is an equilibrium between various oligomers, such as dimers, tetramers, hexamers, and higher aggregates; the dimer is the basic oligomeric unit (159, 216). The dimer is the most active species of the enzyme isolated from *E. coli* (71, 72). The equilibrium between the aggregation states is shifted toward the hexameric form by histidine, by AMP, and by a combination of these ligands, with synergistic effects (73, 161, 216). However, the most powerful ligand for stabilizing the hexameric form is the product, PRATP. Using the purified transferase from *E. coli*, Tebar et al. (265, 266) demonstrated that one of the two substrates of the transferase, PRPP, brings about a dissociation of hexamers and higher aggregates, with a resulting increase in the concentration of dimers. On the other hand, ATP counteracts PRPP in this respect. This finding is in apparent conflict with data indicating that the hexameric form of the homologous enzyme of *S. typhimurium* is stabilized by the substrates (ATP and PRPP) (34). The different behaviors observed for the two transferases may simply reflect differences in the experimental conditions.

The aggregation state of the transferase of *E. coli* has been, at least in part, related to regulation of its activity: ligands that stabilize the hexameric form of the transferase also play an inhibitory role in its activity (71, 72, 161). Feedback control of the transferase by histidine was first documented in 1961 (18). Klungsøyr et al. (160) found that the transferase from *E. coli* is insensitive per se to histidine inhibition; the histidine effect becomes apparent only in the presence of the product of the reaction, PRATP, and is further increased by AMP. The synergistic inhibition by the product of the reaction and the end product of the pathway represents a sophistication of the general principle of feedback control, and it has been found to also regulate the activity of the glutamine synthetase (141). The inhibitory effect of the AMP supports the energy charge theory proposed by Atkinson (160) and seems logical if we consider the high metabolic cost required for histidine biosynthesis.

The product of the reaction of the transferase, PRATP, is hydrolyzed to *N*′-5′-phosphoribosyl-AMP (PRAMP). This irreversible hydrolysis is catalyzed by one of the two activities, corresponding to the carboxyl-terminal domain of the enzyme coded for by the *hisI* gene (255), formerly known as *hisIE* (Fig. 1). The other activity, which is localized in the amino-terminal domain of the bifunctional enzyme (53, 61), is a cyclohydrolase, which opens the purine ring of PRAMP, leading to the production of an imidazole intermediate, the *N*′-[(5′-phosphoribosyl)-formimino]-5-aminoimidazole-4 carboxamide-ribonucleotide (abbreviated 5′-ProFAR, or BBMII) (255).

The fourth step of the pathway is an internal redox reaction, also known as Amadori rearrangement, involving the isomerization of the aminoaldose 5′-ProFAR to the aminoketose *N*′-[(5′-phosphoribulosyl)-formimino]-5-aminoimidazole-4 carboxamide-ribonucleotide (5′-PRFAR, or BBMIII). The reaction is catalyzed by the *hisA* gene product (181, 182, 254).

Although the pathway of histidine biosynthesis was almost completely characterized by 1965, the biochemical event leading to the synthesis of imidazole-glycerol-phosphate (IGP) and 5′-phosphoribosyl-4-carboxamide-5-aminoimidazole (abbreviated PRAIC, AICAR, or ZMP) from the 5′-PRFAR remained ambiguous for a long time. The functions of the *hisH* and *hisF* genes were known to be involved in the overall process in eubacteria (254), but the catalytic properties of each protein had not been completely characterized. Smith and Ames (254) demonstrated that this process required the presence of glutamine as a source of amide nitrogen; however, a high concentration of ammonia could be substituted for glutamine and the *hisH* enzyme at alkaline pH in an in vitro system. These authors suggested that the *hisH* gene product added the amide nitrogen of glutamine to that portion of 5′-PRFAR which cyclized to form IGP at intracellular pHs. The *hisF* gene product (the cyclase) cleaved the side chain of 5′-PRFAR; the cyclization of the moiety cleaved away was supposed to occur spontaneously. It was hypothesized that the *hisF* and *hisH* gene products controlled two separate steps whose order in the pathway was difficult to establish. As a consequence, the structure of the intermediate could not be predicted (185, 254). The last blind spot of histidine biosynthesis has recently been clarified by analyzing the catalytic properties of the *hisH* and *hisF* gene products from *E. coli* (158, 227, 228). Klem and Davisson (158) found that the protein encoded by the *hisF* gene has an ammonia-dependent activity that is responsible for the conversion of PRFAR to AICAR and IGP, while the product of the *hisH* gene had no detectable catalytic properties. However, in combination, the two proteins were able to carry out the reaction in the presence of glutamine as a nitrogen donor without releasing any free metabolic intermediate. The *hisH* and *hisF* gene products formed a stable 1:1 dimeric complex that constituted the IGP synthase holoenzyme. The existence of this functional dimeric complex has been proven genetically by isolation and characterization of a mutated *hisF* gene product in *Klebsiella pneumoniae* which catalyzes the reaction utilizing free ammonia but not the ammonia moiety from glutamine bound to the *hisH* gene product. The mutation, which results in the replacement of aspartic acid by asparagine, has been speculated to affect the interaction between the *hisH* and *hisF* gene products (228). A striking feature of the protein coded for by the *hisH* gene is that, despite the high degree of active-site sequence homology with several amidotransferases which also exhibit glutaminase activity in the absence of their respective substrates (110, 273), its glutamine-dependent catalytic properties require the presence of the *hisF* gene product (158). Another heterodimeric glutamine amidotransferase, the enzyme aminodeoxychorismate synthase, which is composed of the proteins encoded by the *pabA* and *pabB* genes, has similar features (112, 296).

AICAR, which is produced in the reaction catalyzed by the IGP synthase, is recycled into the de novo purine biosynthetic pathway (see below). The other product, IGP, is dehydrated by one of the activities of the bifunctional enzyme encoded by the *hisB* gene, that corresponding to the carboxyl-terminal domain (42). The resulting enol is ketonized nonenzymatically to imidazole-acetol-phosphate (IAP).

The seventh step of the pathway consists of a reversible transamination involving IAP and a nitrogen atom from glutamate. The reaction leads to the production of α-ketoglutarate and L-histidinol-phosphate (HOL-P) and is catalyzed by a pyridoxal-P-dependent aminotransferase encoded by the *hisC* gene (42). This enzyme shares certain mechanistic features with other pyridoxal-P-dependent aminotransferases: (i) covalent binding of pyridoxal-P to an active-site lysine residue; (ii) the formation of an aldimine between pyridoxal-P and the amino acid substrate as the first intermediate; and (iii) a ping-pong Bi-Bi mechanism of catalysis (140, 191).

The HOL-P is converted to L-histidinol (HOL) by the phosphatase activity localized in the amino-terminal domain of the bifunctional enzyme encoded by the *hisB* gene (42).

During the last two steps of histidine biosynthesis, HOL is oxidized to the corresponding amino acid L-histidine (His) (1). This irreversible four-electron oxidation proceeds via the unstable amino aldehyde L-histidinal (HAL), which is not found

as a free intermediate (1, 109). A single enzyme, the L-histidinol dehydrogenase encoded by the *hisD* gene, catalyzes both oxidation steps, probably to prevent decomposition of the unstable intermediate (179). This enzyme represents one of the first established examples of a bifunctional NAD^+-linked dehydrogenase (46, 157). Most of the information about the L-histidinol dehydrogenase comes from the enzyme purified from *S. typhimurium*. It is a homodimeric Zn^{2+} metalloenzyme which functions by carrying out the first oxidation step at an active site on one subunit and then moving the intermediate to a vicinal site on an adjacent subunit (46, 48, 81, 109, 115). Steady-state kinetic patterns demonstrated that the enzyme acts via a Bi-Uni Uni-Bi ping-pong mechanism: HOL binds first to the enzyme, followed by the binding of NAD^+; His is the last product to dissociate (46, 108).

A comparative analysis of the *E. coli* and *S. typhimurium* *hisD* gene products and the homologous region (*HIS4*C) of the multifunctional product of the *Saccharomyces cerevisiae HIS4* gene (80) showed the presence of two long regions characterized by highly conserved amino acid sequences (45). These regions were supposed to represent functional domains of the enzyme: the amino-terminal region responsible for the first oxidation step and the carboxyl-terminal region responsible for the second one. Genetic data on the existence of two groups of mutations in *hisD* which exhibit intracistronic complementation further supported this belief (124).

An essential lysine residue appears to participate in the reversible oxidation-reduction converting the alcohol HOL to the aldehyde HAL during the first step of the reaction (47). From the well-elucidated mechanism of catalysis of the glyceraldehyde-3-phosphate dehydrogenase, in which an active-site cysteine contributes to form a thiohemiacetal intermediate (121), it was assumed that the thiohemiacetal-thiolester pair represented the route for the aldehyde oxidation in the second step catalyzed by the histidinol-dehydrogenase. This hypothesis was initially supported by evidence that the *S. typhimurium* enzyme contains two conserved cysteine residues, Cys-116 and Cys-153, and is inactivated by modification of Cys-116 by the reagent 4-nitro-7-chlorobenzadioxazole (114). However, the recent result that mutant enzymes with either alanine or serine substitution of Cys-116 and Cys-153 are active with kinetic properties resembling those of the wild-type enzyme has ruled out this hypothesis and suggested that the reaction might proceed through a scheme different from those common to most aldehyde dehydrogenases (267).

Metabolic Links between the Histidine and Purine Biosynthetic Pathways: the AICAR Cycle

Mutants bearing nonfunctional enzymatic activities which are required for histidine biosynthesis grow normally in minimal medium when supplied with exogenous histidine. On the basis of this evidence, the pathway was supposed to lack any branch point leading to other metabolites required for growth (15, 42). Nevertheless, the two initial substrates of histidine biosynthesis, PRPP and ATP, play a key role in intermediate and energetic metabolism and link this pathway to the biosynthesis of purines, pyrimidines, pyridine nucleotides, folates, and tryptophan (43, 204, 223). These metabolic links would, at least in part, account for the pleiotropic effects generated by the derepressed synthesis of the enzymes coded for by the histidine operon.

It has been calculated that 41 ATP molecules are sacrificed for each histidine molecule made (42). The considerable metabolic cost may explain the finding that histidine regulatory mutants which constitutively express the histidine operon and lack feedback control of the biosynthetic pathway require adenine for growth at 42°C (152, 252, 260).

The purine and histidine biosynthetic pathways are connected through the AICAR cycle (39). AICAR, a by-product of histidine biosynthesis, is also a purine precursor. This conversion involves a folic acid-mediated one-carbon (C-1) transfer (204). Bochner and Ames (39) reported that the unusual nucleotide 5-aminoimidazole-4-carboxamide-riboside-5′-triphosphate (ZTP) accumulated in *S. typhimurium* cells following treatment thought to lower the folic acid pool and that strains unable to make Z-ribotides were hypersensitive to antifolate drugs. Under the same treatment, ZTP production was also detected in *E. coli* cells, even though the accumulation of the unusual nucleotide did not correlate strictly with folate deficiency (234). Bochner and Ames (39) proposed that ZTP is an alarmone signaling C-1-folate deficiency and mediating a physiologically beneficial response to folate stress. This belief is supported by several findings concerning ZTP synthesis. At variance with other triphosphate ribotides, whose synthesis involves a two-step process controlled by specific monophosphate kinases and a nonspecific diphosphate kinase, ZTP is made by pyrophosphate transfer onto ZMP in a single enzymatic reaction catalyzed by PRPP synthetase (39, 239, 240). The specificity of this conversion is also supported by results from kinetic studies indicating that the increase in the ZTP pool does not track the increase in the ZMP pool (39).

Inhibition of folate metabolism leads to alterations of intracellular processes which may involve ZTP-mediated responses. Interesting effects on gene expression are observed in folate-deficient cells. For example, the availability of 10-formyl-tetrahydrofolate influences the mode of derepression—sequential or simultaneous—of the genes clustered in the *his* operon of *S. typhimurium*, possibly by affecting the mechanism of translation coupling at the intercistronic barriers (37, 42, 219, 220). Addition of inhibitors of folate metabolism induces polarity in *E. coli* and *S. typhimurium* (9, 221) and affects the rate of decay (153) or processing (9) of several polycistronic mRNAs. Other bacteria, such as *Bacillus subtilis*, respond to folate shortage by initiating sporulation (101, 128, 192). The involvement of AICAR as a metabolic regulator in different aspects of bacterial life is also suggested by the fact that 5-aminoimidazole-4-carboxamide-riboside or, more likely, its phosphorylated form, AICAR, is required throughout most of infection thread development during the process of nodule formation by many *Rhizobium* species (205, 206). However, the involvement of ZTP in these processes is only speculative, and the evidence for a folate stress regulon controlled by ZTP remains elusive.

It has been known for a long time that constitutive expression of the *his* operon of *S. typhimurium* results in a number of phenotypic changes: (i) growth inhibition at 42°C that is somehow relieved by methionine (91); (ii) wrinkled morphology of colonies grown either in 2% glucose or on green plates (105, 200, 237); and (iii) growth inhibition in high-salt media (55). A similar pleiotropic response is also observed in *E. coli* (97). The wrinkled morphology of the *his* constitutive strains is due to filament formation as a consequence of cell division inhibition (105, 200). Murray and Hartman (200) demonstrated that the pleiotropic response is caused by the overproduction of the *hisH* and *hisF* gene products acting in a concerted fashion. We now know that these two proteins associate to form the heterodimeric IGP synthase complex, which catalyzes the closure of the imidazole ring of histidine, thereby releasing the by-product AICAR. Since AICAR is a potential precursor of the unorthodox nucleotide ZTP, a proposed alarmone, it was hypothesized that the pleiotropic response might be caused by

the enzymatic activity of IGP synthase leading to AICAR accumulation.

As the base 5-amino-4-imidazole carboxamide is a mutagen in *E. coli* (201), mutagenic properties of the corresponding riboside AICAR or its derivatives were also invoked to account for the wrinkled phenotype by analogy with SOS-induced filamentation upon perturbation of DNA synthesis (79). Isolation of an antimutator strain of *E. coli* carrying a *purB*(Ts) mutation (104) was in agreement with this proposal, since *purB* catalyzes the synthesis of AICAR in the purine biosynthetic pathway (204).

However, more recent evidence has ruled out this hypothesis. First, it has been reported that AICAR is not an endogenous mutagen in *E. coli* (96). Second, overexpression of the *his* operon in *S. typhimurium* cells does not result in increased incidence of spontaneous mutations as a consequence of AICAR accumulation, confirming that the pleiotropic response does not involve DNA damage (94). This conclusion agrees with the observations that overexpression of the *his* operon does not induce the SOS response (105) and that filamentation in *his* constitutive strains is independent of the two SOS-associated division inhibitors, SfiA and SfiC (94). Moreover, the filaments observed in *his* constitutive strains, unlike the aseptate filaments formed after SOS induction, show smooth partial constrictions in DNA-free regions (97) and are reminiscent of those observed in *ftsI*(Ts) mutants bearing a temperature-sensitive penicillin-binding protein 3 (PBP 3) (264). Third, elevated levels of IGP synthase cause inhibition of cell division by themselves and not via AICAR production. Filamentation, as well as the other pleiotropic effects associated with *his* overexpression, was shown to occur even in *E. coli* and *S. typhimurium* strains devoid of AICAR as a consequence of interrupting the carbon flow through the histidine and purine pathways in *his pur* double mutants (94, 97).

From intergenic suppression analysis, it has been suggested that elevated levels of the *hisH* and *hisF* gene products induce filamentation by interfering somehow with synthesis of the cell wall (21, 22). Several of these suppressors result in spherical cells with increased autolysis and sensitivity to penicillin or related antibiotics. One such mutant was affected at the *envB* locus, also known as *mre* in *E. coli* (22). Interestingly, an *E. coli mreB* mutant, which also has spherical shape and is hypersensitive to mecillinam, overproduces PBP 3 (279). Altogether, these findings suggest that division inhibition in *his* constitutive strains may result from a shortage of septal murein synthesis catalyzed by PBP 3 (97). The classical approach of studying intergenic suppression has recently led to the identification of four novel loci on the *S. typhimurium* chromosome: *osmH*, *sfiW*, *sfiX*, and *sfiY*. These suppressors behave as either "general" or "partial" suppressors of the pleiotropic response. Epistatic effects among suppressors have been also documented and suggest a pathway-like model for the *his* constitutive pleiotropic response (93).

It is conceivable that further study of the pleiotropic response triggered by the overexpression of *hisH* and *hisF* gene products will facilitate the discovery of genes controlling the metabolic pathway(s) leading to cell division in *E. coli* and *S. typhimurium.*

ORGANIZATION OF THE HISTIDINE GENES

Since the beginning of microbial genetic studies, histidine-requiring mutants were frequently isolated and characterized. These works led to the identification of all the genes necessary for histidine biosynthesis in *E. coli* and *S. typhimurium* and to their localization on the genetic map (289). Similar work on the yeast *S. cerevisiae* also allowed the identification and genetic mapping of several genes involved in histidine biosynthesis (197). After the introduction of DNA sequencing techniques and genetic engineering in microbial genetics, the data on *his* genes accumulated rapidly for model microorganisms and for several other bacterial and fungal species, providing a wealth of information on at least 14 bacterial (including Archaea), 5 fungal, and 3 plant species, with more than 60 genes sequenced. Table 1 reports the complete list of all these genes, grouped according to their homology with the well-characterized genes of the *his* operon of *E. coli*. Many of these genes/operons were first identified by the ability of cloned DNA fragments to complement histidine auxotrophic mutations either in *E. coli* or in homologous hosts. Final identification was generally achieved from DNA and protein sequence comparison with the *E. coli* counterparts, assuming, as it is widely accepted, that the biosynthetic pathway is fundamentally the same in all organisms.

As indicated by the frequent occurrence of genetic complementation, homologous genes (which are presumed to code for proteins performing the same function in the biosynthetic pathway) in different species are generally similar. The similarity in the overall lengths of the genes extends to the molecular weights and secondary structures of the proteins encoded.

In many of the species in which *his* genes were identified and characterized, they were not found interspersed in the genome but clustered with other genes to constitute complete operons or at least part of them. The same is in part true for operonless fungi, in which some of the *his* genes resulted from the fusion of different parts, each of which is homologous to different bacterial genes. The organization of genes in the *his* operons or clusters is variable among the different species, indicating that during evolution, genes were separated or linked, apparently without severe constrains (see below). In other bacterial operons which have been characterized in several species, such as the *trp* operon, gene order was mostly found to be invariant (69). The organization of the known *his* gene clusters in microorganisms is presented in Fig. 2.

Eubacteria

Gram-negative bacteria. In the *Enterobacteriaceae* members *E. coli* and *S. typhimurium*, a single operon composed of eight genes very tightly linked to each other encodes all the enzymes required for the biosynthesis of histidine. The complete genetic structure of the *E. coli* and *S. typhimurium* operons was reported by Carlomagno et al. (53), with minor differences found in genes *hisG* and *hisD* by Jovanovic et al. (154). The operons measure 7,389 and 7,438 bp in *E. coli* and *S. typhimurium*, respectively, with an overall homology of 81%. The order of the genes in the operon does not match the sequence by which the enzymes they encode take part in the synthesis of histidine (Fig. 1); it is possible that the particular gene order resulted from both regulatory and metabolic constraints (see below, Evolution of *his* clusters). In the other member of the *Enterobacteriaceae* for which partial DNA sequences are available, *K. pneumoniae* (228, 233), the data on a part of *hisG*, the complete *hisF*, and a very small part of *hisA* and *hisI* suggest a general organization similar to that of *E. coli* and *S. typhimurium.*

In the gram-negative proteobacterium *Azospirillum brasilense*, belonging to the α-purple subdivision, an operon structure with a transcription initiator, seven open reading frames (ORFs), and a transcription terminator was found (83, 85). Only five of these ORFs, however, are homologous to *E. coli* genes; the remaining two code for proteins without any ho-

TABLE 1. Histidine genes in different prokaryotic and eukaryotic organisms

Gene	Organism	Sequence[a] (database)	Length (bp)	Enzyme coded or homologous enzyme	No. of residues/ mol wt	Comple-mentation[b]	Reference(s) or accession no.
hisA	*E. coli*	ECHISOP (G)	735	Phosphoribosyl-formimino-5-amino-1-phosphoribosyl-4-imidazole carboxamide isomerase (EC 5.3.1.16)	245/26,030		53
	S. typhimurium	STHISOP (G)	738		246/26,197		53
	A. brasilense	ABHISHAFE (G)	762		254/26,788	+E.c.	83
	S. coelicolor	SCHISOPA (E)	720		240/25,097		176
	L. lactis	LACHISBIO (G)	717		240/25,097		77
	M. voltae	MVOHISA (G)	726		242/26,739	+E.c.	70
	M. vannielii	MVHISA (E)	714		238/25,709	+E.c.	70
	M. thermolithotrophicus	MVOHISAB (G)	714		238/25,649	+E.c.	283
	S. cerevisiae (*HIS6*)		783		261/29,554	+E.c.	84
hisB	*E. coli*	ECHISOP (G)	1,068	Imidazoleglycerol-phosphate dehydratase (EC 4.2.1.19); histidinol-phosphate-phosphatase (EC 3.1.3.15) (bifunctional)	356/40,283		53
	S. typhimurium	STHISOP (G)	1,065		355/40,138		53
*hisB*d	*A. brasilense*	ABHISBDH (G)	621	Imidazoleglycerol-phosphate dehydratase (EC 4.2.1.19)	207/22,664	+E.c.	85
	S. coelicolor	SCHISOPA	591		197/21,588	+E.c.	176
	L. lactis	LACHISBIO (G)	600		200/22,005	+E.c.	77
	Anabaena sp.	ASBIFA (E)	627		209/22,890		262
	S. cerevisiae (*HIS3*)	SCHIS3G (E)	657		219/23,788		261
	S. kluyveri (*k-HIS3*)	SKHIS3G (E)	696		232/25,325	+S.c.	284
	T. harzianum	THIGMPR (E)	627		208/22,356	+S.c.	107
	S. pombe	SPU07831 (G)	648		216	+S.c.	U07831
	P. parasitica	PPHIS3G (G)	1,356		452/47,961		Z11591
	T. aestivum	TA02690	585 (inc)[c]		195/21,250		P34048
	A. thaliana	AT02689 (E)	810		270/29,225		P34047
*hisB*px	*S. cerevisiae* (*HIS2*)	SCU09479 (G)	1,008	Histidinol-phosphate-phosphatase (EC 3.1.3.15)	335		180
hisC	*E. coli*	ECHISOP (G)	1,071	Imidazole acetol phosphate aminotransferase (EC 2.6.1.9)	356/39,339		53
	S. typhimurium	STHISOP (G)	1,077		359/39,726		53
	Z. mobilis (*hisH*)	ZMTYRC (E)	1,110		370/40,631	+E.c.	116
	A. pasteurianus (*his1*)	ABCHPA (G)	1,071		356		263
	M. smegmatis	MSHISCD (G)	657 (inc)		219/23,343		134
	S. coelicolor	SCHISOPA (G)	1,107		369/40,352		176
	L. lactis	LACHISBIO (G)	1,080		360/41,448	+E.c.	77
	B. subtilis (*hisH*)	BSHISHA (E)	1,089		363/40,414		129
	H. volcanii	HALHPA (G)	1,083		361/39,415	−E.c.[d]	67
	S. cerevisiae (*HIS5*)	SCHIS5	1,155		385/42,542		209
	S. pombe (*his3*)	SPU07830 (G)	1,451[e]		384/42,733		49
	C. maltosa	CMCHIS5	1,923		640		130
hisD	*E. coli*	ECHISOP (G)	1,302	Histidinol dehydrogenase (EC 1.1.1.23) (bifunctional)	434/46,199		53, 154
	S. typhimurium	STHISOP (G)	1,299		433/45,823		53
	L. lactis	LACHISBIO (G)	1,290		430/47,350	+E.c.	77
	S. coelicolor	STMHISHOPA (G)	1,323		441		177
	M. smegmatis	MSHISCD (G)	1,338		445/46,702		134
	P. pastoris	PPHIS4G	2,534		844		68
	S. cerevisiae (*HIS4*)	YSCHIS4 (G)	2,397		799/87,935		80
	N. crassa (*his-3*)	NCHIS3 (E)	2,648[f]		863/94,658		174
	B. olearacea	BOHDHQ	1,402		438/47,474[g]		202
hisF	*E. coli*	ECHISOP (G)	774	Cyclase	258/28,452		53
	S. typhimurium	STHISOP (G)	774		258/28,366		53
	K. pneumoniae	KPHISFCY (G)	776		242		228
	A. brasilense	ABHISHAFE (G)	783		261/28,059	+E.c.	83
	L. lactis	LACHISBIO (G)	732		244/26,659	+E.c.	77
	S. cerevisiae (*HIS7*)	SCHIS7 (E)	1,656		552/61,082		164
hisG	*E. coli*	ECHISOP (G)	897	ATP phosphoribosyl transferase (EC 2.4.2.17)	299/33,264		53, 154
	S. typhimurium	STHISOP (G)	897		299/33,209		53
	L. lactis	LACHISBIO (G)	624		208/23,570	+E.c.	77
	S. cerevisiae (*HIS1*)	SCHI01 (E)	891		297/32,263		133
	K. pneumoniae	KPHISG (G)	300 (inc)		100/11,408		232

Continued on following page

TABLE 1—*Continued*

Gene	Organism	Sequence[a] (database)	Length (bp)	Enzyme coded or homologous enzyme	No. of residues/ mol wt	Comple-mentation[b]	Reference(s) or accession no.
hisH	*E. coli*	ECHISOP (G)	588	Glutamine amidotransferase	196/21,651		53
	S. typhimurium	STHISOP (G)	582		194/21,520		53
	A. brasilense	ABHISHAFE (G)	576		192/20,824		83, 85
	S. coelicolor	STMHISHOPA (G)	666		222/23,841		176
	L. lactis	LACHISBIO (G)	606		202/22,438		77
	S. cerevisiae (*HIS7*)	SCHIS7 (E)	1,656		552/61,082		164
hisI	*E. coli*	ECHISOP (G)	612	PR-ATP pyrophosphohydrolase:PR-AMP cyclohydrolase (EC 3.5.4.19) (bifunctional)	204/22,760		53
	S. typhimurium	STHISOP (G)	609		203/22,663		53
	K. pneumoniae		60 (inc)		20		228
	L. lactis	LACHISBIO (G)	606		212/24,673	+E.c.	77
	S. cerevisiae (*HIS4*)	YSCHIS4 (G)	2,397		799/87,935		80
	N. crassa (*his-3*)	NCHIS3 (E)	2,648[f]		863		174
	P. pastoris	PPHIS4G	2,534		844		68
hisI	*M. vannielii*	MVHISI (G)	408	PR-AMP cyclohydrolase	136/15,619	+E.c.	32
hisE	*A. brasilense*	ABHISHAFE (G)	336	PR-ATP pyrophosphohydrolase	111/11,944	+E.c.	83
orf168	*A. brasilense*		504		168/18,459		83
orf122	*A. brasilense*		366		122/13,244		83
orf1	*S. coelicolor*		162		54/5,685		176
orf2	*S. coelicolor*		228 (inc)		76/7,985		176
orf3	*L. lactis*		984	Histidyl-tRNA synthetase	328/37,908		77
orf6	*L. lactis*		789		263/30,701		77
orf8	*L. lactis*		786	Apha3 enzyme	262/30,706		77
orf13	*L. lactis*		807		269/31,346		77
orf91	*M. voltae*		273		91		70
orf294	*M. voltae*		882		294		70
orf29	*M. vannielii*		87		29/3,255		32
orf634	*M. vannielii*		1,902		634/71,094		32
orf150	*M. vannielii*		450		150		70
orf547	*M. vannielii*		1,641		547		70
orf114	*M. thermolithotrophicus*		342		114		283
orf145	*M. thermolithotrophicus*		435		145		283
orf206	*M. thermolithotrophicus*		618		206		283

[a] Gene name in the data banks. E, EMBL; G, GenBank.
[b] Complementation (+) or noncomplementation (−) of *E. coli* (E.c.), *S. typhimurium* (S.t.), or *Saccharomyces cerevisiae* (S.c.) homologous mutants.
[c] inc, sequence available is incomplete.
[d] The corresponding *E. coli* gene complements the *H. volcanii* mutation.
[e] The gene is interrupted by three introns.
[f] The gene is interrupted by one 59-bp-long intron.
[g] Size of the mature protein after processing.

mology to other *his* genes. Assuming that the histidine biosynthetic pathway of *A. brasilense* is identical to that of *E. coli*, there are at least three genes missing in the operon, *hisGDC*. A fourth missing gene could be the sequence coding for the PRATP pyrophosphohydrolase/PRAMP cyclohydrolase, *hisI* of *E. coli*, whose corresponding ORF in *A. brasilense* (*hisE*) is just a part of it. The same deduction can be applied to the *hisB*px moiety of the *E. coli hisB* (see below) that is also missing in the *A. brasilense* operon. The presence of other *his* genes in the *A. brasilense* genome unlinked to the main operon is further suggested by the inability of the genes of the identified cluster to complement some uncharacterized histidine-requiring mutants of *A. brasilense* (87). The order of genes in the *A. brasilense his* operon, *hisB*d*HAFE*, is the same as in *E. coli*. The same gene order does not mean, however, the same gene organization: in fact, one of the two unidentified ORFs lies between *hisH* and *hisA*, another unidentified ORF at the end of the operon separates *hisE* from the terminator, and finally *hisB*d and *hisE*, as already mentioned, correspond to just a portion of the *E. coli* genes (see below).

Gram-positive bacteria. A single operon organization was also found in the gram-positive bacterium *Lactococcus lactis*, in which 12 ORFs have been identified (77). Eight ORFs are homologous to the *E. coli his* genes, indicating that all the enzymes of the pathway are coded for by this operon. The exception is the product of the *hisB* gene, which, like in other organisms (see below), is only present with the dehydratase activity coded by the *hisB*d portion of the gene, while *hisB*px, the phosphatase domain, is apparently absent. Four ORFs code for unidentified proteins with unknown functions. The order of the genes is similar to that in *E. coli* in the 3′ portion of the operon for the *hisHAFI* genes. The 5′ portion of the operon apparently underwent a translocation with respect to the *E. coli* arrangement, moving *hisC* from immediately downstream of *hisD* to upstream of *hisG*, at the very beginning of the operon (Fig. 2). The general organization of the *his* operon of *L. lactis* reflects the features of other genes for amino acid biosynthesis in lactic acid bacteria, such as the single chromosomal location and the presence of unrelated ORFs (62).

Clustered genes for histidine biosynthesis were also found in

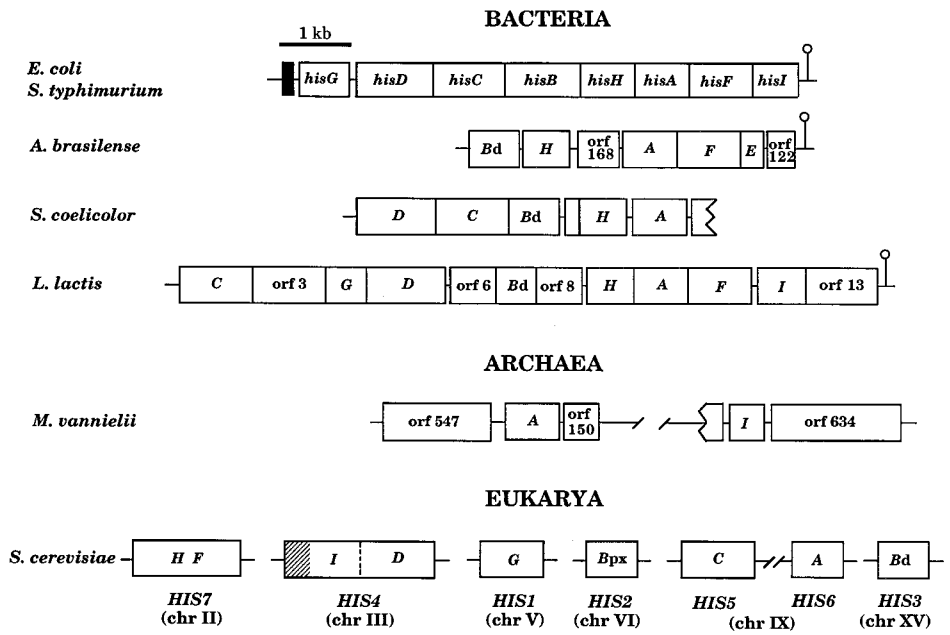


FIG. 2. Organization of some of the histidine biosynthetic genes sequenced to date in different organisms. The single gene encoding a bifunctional enzyme, formerly known as *hisIE* (289), has been renamed *hisI* in *E. coli* (27). In this review, we have therefore used *hisI* for organisms with a single gene and *hisI* and *hisE* for organisms with two independent genes. Another gene encoding a bifunctional enzyme, *hisB*, is often split into two separate genes in different organisms. We refer to them as *hisB* proximal (*hisB*px), encoding the HOL-P phosphatase, and *hisB* distal (*hisB*d), encoding the IGP dehydratase. Solid box, leader sequence; stem and loop, transcription terminators. Open boxes in the *S. coelicolor* cluster indicate ORFs with unknown function. Lines between genes indicate intergenic regions. Hatched box, 5′-proximal region of the *HIS4* gene encoding a sequence not homologous to known *his* genes. chr, chromosome.

the gram-positive organism *Streptomyces coelicolor*, in which the *his* genes were mapped in three unlinked loci (137). Two minor loci, each containing one gene, are located at 2 o'clock and 6 o'clock on the strain A3(2) chromosome (the latter was identified as *hisB*px). The third locus, mapping at 12 o'clock, contains the main *his* cluster, with seven ORFs, five of which are homologous to *E. coli hisDCB*d*HA* (176, 177). Also in *S. coelicolor*, the general gene order is the same as in *E. coli*, with the insertion of unknown ORFs changing the organization of the operon. In this case, however, the sequence of the last ORF at the 3′ end of the cluster was not completely determined, and it is possible that other ORFs are included in the same operon. It is interesting that three of the seven ORFs start with unusual codons: *hisC* and *hisD* with GUG, and *hisH* with UUG. However, GUG at least was found to be a rather frequent start codon in *Streptomyces* spp. (136).

In two other gram-positive bacteria, *Mycobacterium smegmatis* and *B. subtilis*, only limited sequences of *his* genes are available, but it is possible to infer that *M. smegmatis his* genes have the same organization as those of the more closely related *S. coelicolor* (134), with an operon structure starting with *hisD* followed by *hisC*. The *B. subtilis hisH* gene, which is homologous to *E. coli hisC*, seems, on the contrary, to be an isolated gene (129). Another *his* locus, containing all the other *his* genes, was mapped in the *B. subtilis* chromosome, but none of these genes has been sequenced (19). A genetic organization similar to that of *B. subtilis* was postulated for the *his* genes of the related gram-positive organism *Staphylococcus aureus* (217).

Archaebacteria

The *his* genes in archaebacteria are less well known than in eubacteria; only three *his* genes have been recognized in just four species, three of them belonging to the genus *Methanococcus* and one to *Halobacterium* (Table 1). In *M. vannielii*, *M. voltae*, and *M. thermolithotrophicus* (70, 283) a gene highly homologous to *E. coli hisA* has been sequenced together with its flanking regions; the analysis of these sequences failed to demonstrate the occurrence of an operon-like structure. The ORFs surrounding *hisA* are, in fact, apparently unrelated to histidine biosynthesis. On the other hand, no promoter-like sequence was recognizable upstream of *hisA*, although a putative ribosome-binding site was noted (283). A second *his* gene, *hisI*, sequenced in *M. vannielii* (32) was found to be unlinked to *hisA*. If further studies eventually demonstrate an operon structure for the archaebacterial *his* genes, it will contain unrelated ORFs, like the operons found in *L. lactis* and *A. brasilense*. A conserved genetic organization, if not an operon, among archaebacterial *his* genes is suggested by the high level of homology found between the peptides coded for by ORF547, ORF294, and ORF114 preceding *hisA* and between ORF150 and ORF125 following the same gene in the three methanococci. The remaining ORFs do not show any kind of homology to each other. The *Halobacterium volcanii hisC* gene is probably a single gene, as the flanking sequences do not indicate the presence of other *his* genes (67).

Eukaryotes

In eukaryotes, the general rule that no operon structures have been found also applies to the *his* genes. In particular, in the well-known yeast *S. cerevisiae*, the seven genes responsible for the biosynthesis of histidine are located on six different chromosomes (197). Despite the spreading of the *his* genes, a particular sort of gene organization could be seen on two of the yeast *his* genes. *HIS4* (80) and *HIS7* (164) are in fact very large genes whose sequence is homologous to that of two pairs of *E. coli* genes, *hisI* and *hisD* for *HIS4* and *hisH* and *hisF* for *HIS7* (Fig. 2 and Table 1). The multifunctional structure of yeast

HIS4 is also found in *Neurospora crassa his-3* (174) and *Pichia pastoris* (68), as reported below. A gene that complements an *S. cerevisiae HIS4* deletion was also found in *Candida albicans* but has not been sequenced (11). Several other *his* genes have been cloned and sequenced in eukaryotes other than *S. cerevisiae* (Table 1); however, they are single genes, and it is not possible to make inferences about their organization.

Structure of the *his* Genes and Their Products

The high level of homology between corresponding genes for histidine biosynthesis in different organisms indicates that this metabolic pathway was fundamentally conserved during evolution (see below). Looking at the structure of the *his* genes, however, some interesting peculiarities become evident.

hisG. *hisG* genes code for the first enzyme in the biosynthesis of histidine, a protein of about 300 amino acids in *E. coli*, *S. typhimurium*, and *S. cerevisiae* but shorter (about 200 amino acids) in *L. lactis*. Because the short *L. lactis* gene is able to complement *E. coli hisG* mutants (77), it is likely that a considerable portion of the enterobacterial and yeast protein is dispensable for enzymatic activity.

hisB. Two enzymatic activities have been recognized to be coded by the *hisB* gene of *E. coli* and *S. typhimurium* (see above). In other microorganisms studied, on the contrary, the two activities appear to be coded for by two independent genes, *hisB*d, coding for IGP dehydratase, and *hisB*px, coding for HOL-P phosphatase. *hisB*d genes with different levels of homology to each other and with protein products ranging from 195 to 270 amino acids were found in almost all species, including cyanobacteria, fungi, and plants (Table 1). On the other hand, a *hisB*px homologous gene is yet to be found in any other microorganism except *S. coelicolor* (137), in which it is called *hisD* and maps outside of the main gene cluster (and has not been sequenced yet), and *S. cerevisiae* (180), whose *HIS2* is located on chromosome VI, very close to a recombination hotspot. Surprisingly, we did not find a significant degree of similarity with the enterobacterial *hisB* 5′-proximal domain. This finding suggests that dissimilar phosphatases encoded by genes unrelated to the *hisB* 5′-proximal domain can carry out the dephosphorylation of HOL-P and that these genes are either unlinked to the other *his* genes or encoded (77) by some of the uncharacterized ORFs.

A different *hisB*d gene structure was found in the fungus *Phytophtora parasitica*, in which the sequence coding for IGP dehydratase, recognized by the high level of homology with the corresponding protein of *E. coli*, is fused with a DNA fragment whose translation gives an amino acid sequence that is not homologous to that of any of the known histidine biosynthetic enzymes (87).

hisI* and *hisE. The *E. coli*, *S. typhimurium*, and *L. lactis hisI* genes (53, 77), mapping at the ends of their respective operons, also code for a bifunctional enzyme that is responsible, with its amino-terminal domain, for the third step and, with the carboxyl-terminal domain, for the second step of the histidine pathway. This is one example of the lack of correspondence between the order of genes (and coding sequences) in the *his* operon and the order of the biosynthetic steps performed by the enzymes encoded by these genes.

The same enzymatic steps are achieved in fungi, *S. cerevisiae*, *N. crassa*, and *P. pastoris* (68, 80, 174) by a large multifunctional protein whose amino-terminal domain has good homology with the bifunctional *hisI* gene product. The presence of pyrophosphohydrolase and cyclohydrolase activities in the same polyfunctional enzyme is not, however, a universal phenomenon. In the gram-negative organism *A. brasilense* (83) and in the archaebacterium *M. vannielii* (32), the two enzymatic activities seem to be separated between two proteins, coded for by different genes, although in *A. brasilense*, only *hisE* (pyrophosphohydrolase) and, in *M. vannielii*, only *hisI* (cyclohydrolase) have been found up to now.

hisD. The product of the *hisD* gene is likewise a protein with bifunctional activity, histidinol dehydrogenase, performing the last two steps of histidine biosynthesis. Unlike *hisB* and *hisI*, *hisD* is a single unique gene in the nine species in which it has been found and sequenced, including the plant *Brassica olearacea*. In this plant, the gene product, which complements the corresponding *E. coli* mutation, is probably located in the plastid, as indicated by a 31-amino-acid signal peptide typical of proteins transferred to the plastid compartment that is absent in the mature enzyme (202). In the fungi *S. cerevisiae*, *N. crassa*, and *P. pastoris* (68, 80, 174), the two enzymatic activities are carried out by the carboxyl-terminal domain of the same huge protein that also contains the homolog of the *hisI* gene product. The bifunctional enzymatic activity of the *hisD* gene product seems to be a universal property, suggesting that a specific constraint, related to the mechanism of the reaction, favored the persistence of the single-protein arrangement.

The four enzymatic activities, pyrophosphohydrolase, cyclohydrolase, and HOL-dehydrogenase (both steps), are thus performed in fungi by a large superenzyme of 800 to 850 amino acids whose genes appear highly homologous to each other in *S. cerevisiae*, *P. pastoris*, and *N. crassa*, with the only difference in the last species being a 59-bp intron in the region coding for the domain corresponding to the *hisD* product. This intron makes the *N. crassa* gene unable to complement *E. coli hisD* mutations.

hisA-hisF* and *hisH. The *hisA* and *hisF* genes, as reported below, have high sequence homology and probably arose from a gene duplication event. Their gene products participate in two successive steps of the biosynthetic pathway. The two genes are also adjacent in almost all eubacterial operons.

As reported before, the products of the *E. coli hisH* and *hisF* genes in vivo form a heterodimeric enzyme, IGP synthetase, that accomplishes the fifth step of histidine synthesis (158). In prokaryotic operons, however, these genes are always separated by at least a third ORF (Fig. 2). In *S. cerevisiae*, on the other hand, there is a single polypeptide fulfilling IGP synthetase activity coded for by a single gene, *HIS7* (164). The amino acid sequence of this protein shows a high level of homology, in its amino-terminal domain, with the product of *E. coli hisH* and, in the carboxyl-terminal domain, with the product of *E. coli hisF*. These two domains of *HIS7* are separated by a short sequence of 22 codons whose product lacks homology to either the *hisH* or *hisF* gene product. The portion of the *S. cerevisiae HIS7* sequence corresponding to the eubacterial *hisF* gene also encodes a peculiar structure in the extreme amino-terminal end of the protein product. In this part of the protein, the homology with the *hisF* product is partially lost because of six short nonhomologous sequences that render this domain of the yeast protein almost one-third longer than the bacterial counterpart. Assuming that the catalytic activity of HisF resides in the carboxyl part of the polypeptide while the interaction with HisH is brought about by the amino part, a number of sequence alterations in the yeast homolog are explicable as the loss of interactive sites which are no longer necessary since *hisH* and *hisF* are fused in a single gene, *HIS7* (87). This hypothesis is strongly supported by the recent finding of Rieder et al. (228), who suggested that a mutation falling in the F1 module of the *K. pneumoniae hisF* gene probably affects the interaction between HisH and HisF.

ORFs. Figure 2 reports the structure of DNA regions of

different organisms in which histidine biosynthetic genes were found to be more or less grouped together. A remarkable feature of these (bacterial and archaebacterial) clusters is the presence of several ORFs with unknown function: 17 ORFs have been identified between or flanking *his* genes in *A. brasilense*, *S. coelicolor*, *L. lactis*, *M. vannielii*, *M. voltae*, and *M. thermolithotrophicus*. The involvement of these ORFs in the biosynthesis of histidine is an open question, as their function has not been determined and mutants for studying the phenotype are not available. The only connection of these ORFs with histidine biosynthesis is their linkage with the *his* genes. Furthermore, information derived from sequence analysis has not been of much help. In *L. lactis*, four unknown ORFs scattered throughout the operon were detected. One of them, ORF3, is homologous to a known gene, *E. coli hisS*, coding for histidyl-tRNA synthetase (77); however, the *L. lactis* ORF lacks an essential motif implicated in synthetase activity (99), suggesting that it has a role different from tRNA aminoacylation. What the function, if any, of this gene could be is not clear, although a regulatory role in histidine biosynthesis was postulated (77). ORF8 is partially homologous to Apha3 enzyme, which inactivates aminoglycoside antibiotics (272), but does not seem to be active for this function. Delorme et al. (77) hypothesize that ORF8 could carry out dephosphorylation of HOL-P, the function of the missing *hisB*px gene product. The remaining two ORFs were not homologous to any known gene sequenced.

Two ORFs of unknown function were found in the *his* operon of *S. coelicolor* (176), one very short and the other at the end of the sequenced region. Neither of these ORFs shows homology to known genes.

Two ORFs were likewise detected in the *his* operon of *A. brasilense*. The protein coded for by the first ORF showed significant homology with the product of the *E. coli hisG* gene, although the homology is limited to the 20 amino-terminal amino acids out of the 168 forming the putative protein. Since the homolog of *E. coli hisG* has not yet been found in *A. brasilense*, the possibility that the product of ORF168 accomplishes the first step of histidine biosynthesis cannot be excluded. The second *A. brasilense* unknown ORF (ORF122) is localized at the end of the operon, just before the transcription terminator. The gene product of this ORF was found (87) to be homologous with two apparently unrelated proteins: cow IPCK-1, inhibitor of protein kinase (198), and a protein from a cyanobacterium (*Synechococcus* sp.) with unknown function (50). These homologies, however, do not provide any convincing explanation for the function of the putative protein coded for by ORF122.

In the three *Methanococcus* species for which *his* genes have been sequenced, they are surrounded by nine unknown ORFs. None of these ORFs or their products show homology with known genes. Some of them seem to be partially homologous to each other (283); these are ORF547, ORF294, and ORF114, which lie upstream of *hisA*, and ORF150 and ORF145, which lie downstream of *hisA*. ORF206 appears to be deleted from the genome of the two mesophilic *Methanococcus* species, with only a few nucleotides in the intergenic region upstream of the *hisA* of *M. voltae* and *M. vannielii* left as witness to the deletion event (283). Whether the abundance of unknown ORFs among the *Archaebacteria* is just a consequence of a different, and still unexplained, histidine biosynthetic pathway or it is a common feature of archaebacterial clusters (operons) to be interrupted by unrelated genes is not known.

Overlapping genes. The analysis of *E. coli* and *S. typhimurium* sequences revealed that almost all the *his* cistrons of the operons have overlapping termination and initiation signals for translation (53). In particular, the stop codons of *hisD*, *hisC*, *hisB*, and *hisH* overlap by one or four bases the ATG of the following gene, while the last three cistrons (*hisA*/*hisF* and *hisF*/*hisI*) overlap by 19 and 7 bases, respectively. Therefore, the ribosome-binding sites of these genes lie inside the preceding cistron. This kind of organization, which was found in other polycistronic operons, is supposed to be associated with the occurrence of translational coupling, a mechanism by which ribosomes start translating a new gene without moving away from mRNA after terminating the translation of the preceding one (163).

The exceptions to overlapping are represented by the regions between the leader peptide and *hisG* and between *hisG* and *hisD*. This latter intercistronic sequence is composed of five nucleotides in *E. coli* and of 102 nucleotides in *S. typhimurium*. The unusually long *S. typhimurium* sequence was analyzed in detail (53) and shown to contain a kind of REP (repetitive extragenic palindromic) sequence, whose function is possibly connected to the regulation of gene expression at the posttranscriptional level (33).

Overlapping cistrons were also detected in the *his* operons of *A. brasilense* (83), *S. coelicolor* (177), and *L. lactis* (77). In these organisms, however, the overlapping of stop and start signals is not a general phenomenon as it is in the *Enterobacteriaceae*; in particular, intergenic regions were mostly detected where ORFs with unknown function were present (Fig. 2). The meaning of this discrepancy, of either regulatory or evolutionary origin, is not known at present.

The compactness of the *his* operon structure, particularly those of *E. coli* and *S. typhimurium*, could somehow be related to the regulation of operon expression at the translational level through translation coupling (213). Experimental evidence, however, did not support this hypothesis.

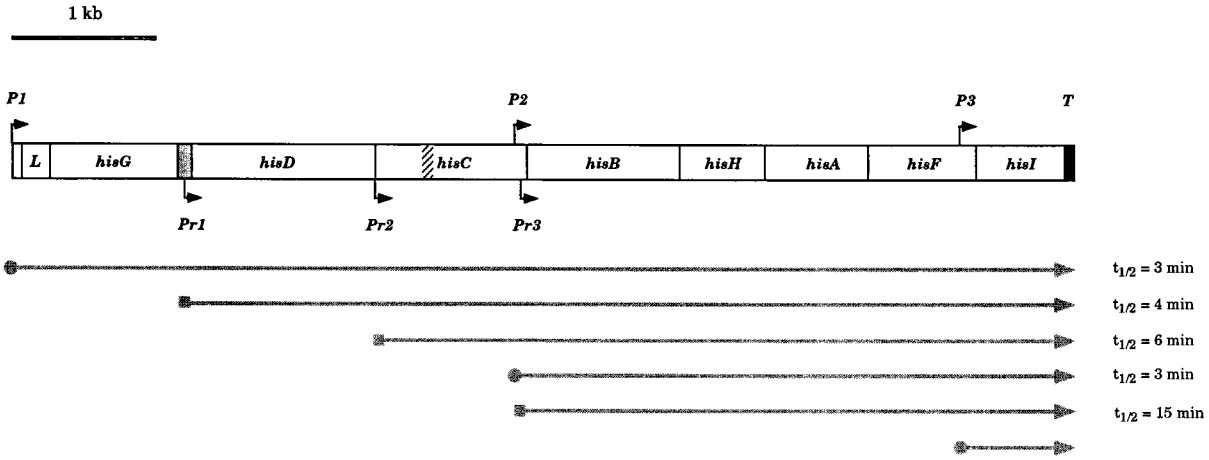
Gene order. As already remarked, the order of the genes in the *his* clusters or operons does not coincide with the order of the enzymatic steps carried out by the proteins that they code for. Moreover, the order of the genes is not rigorously comparable. By observing the eubacterial clusters, it is possible to recognize that some genes tend to remain together in the same order. This is particularly apparent for the four genes *hisBHAF* and partially also for the pair *hisDC*. The tendency of these genes to form stable clusters can be related to the particular role of their products in the biosynthetic pathway. The evolutionary history of these genes and of these particular enzymes and their significance in the evolution of the *his* operon will be discussed in a following chapter.

REGULATION OF HISTIDINE BIOSYNTHESIS

The considerable metabolic cost required for histidine biosynthesis accounts for the evolution in different organisms of multiple and complex strategies to tune finely the rate of synthesis of this amino acid to the changeable environment.

In *S. typhimurium* and in *E. coli*, the biosynthetic pathway is under the control of distinct regulatory mechanisms which operate at different levels. Feedback inhibition by histidine of the activity of the first enzyme of the pathway (see above) almost instantaneously adjusts the flow of intermediates along the pathway to the availability of exogenous histidine. Transcription attenuation at a regulatory element, located upstream of the first structural gene of the cluster, allows coordinate regulation of the amounts of the histidine biosynthetic enzymes in response to the levels of charged histidyl-tRNA (25, 38, 289).

In addition to histidine, the system is also regulated by other



P1 **ttgctt**taaggcgtaaaagtggtt**taggtt**aaaaggtAT

Pr1 Heterogeneous 5' ends at multiple RNase E cleavage sites

Pr2 Heterogeneous 5' ends at multiple RNase E cleavage sites

P2 **tttaaa**tccttatgggatcaggg**cattat**cttAC

Pr3 5'-gcggcugccugcggauuacggucggcacccg**cca**ggaaaaccagccggucauugacgccuuacgugc**ggag**ccagu**auga**

P3 gcgtgccgctgatcgcctccggcggcgcgggcacgatggaacactttctTG

FIG. 3. Transcriptional map of the *S. typhimurium his* operon. Top: the operon is represented as in Fig. 1. The hatched area within *hisC* defines the RNase E target sites. Arrows above the operon indicate the relative positions of *P1* (primary promoter) and *P2* and *P3* (internal promoters); arrows below the operon indicate the relative positions of the three processed (*Pr1*, *Pr2*, and *Pr3*) species. Middle: arrows indicate the limits and extent of the initiated (starting with a circle) and processed (starting with a square) transcripts. The half-lives of individual species are indicated on the right (5, 9). Bottom: DNA sequences of the promoter regions and RNA sequences and/or features of the 5′ regions of the processed transcripts (5, 10, 53). The −35 and −10 putative consensus sequences are in boldface. Nucleotides corresponding to the 5′ ends of the transcripts are in capital letters. The stem-loop structure at the 5′ end of the processed *Pr3* species is indicated by convergent arrows. The CCA consensus, the ribosome-binding site, and overlapping stop and start codons of *hisC* and *hisB* are in boldface.

molecules whose levels are indicative of the energetic and metabolic state of the cell. It has been previously mentioned that PRPP and ATP stimulate the activity of the first enzyme of the pathway, whereas AMP enhances the inhibitory effect of histidine on this enzyme. Moreover, the alarmone guanosine 5′-diphosphate 3′-diphosphate (ppGpp), which is the effector of the stringent response (56), positively regulates *his* operon expression by stimulating transcription initiation under conditions of moderate amino acid starvation and in cells growing in minimal medium (25, 56, 289).

Transcription of the *his* operon is also modulated by a nonspecific mechanism operating during the elongation step at the level of intracistronic rho-dependent terminators (6, 9). These regulatory elements account for the polar phenotype exhibited by several nonsense and frameshift mutations (2, 6, 8, 64, 65). Their physiological significance should be to prevent further elongation of infrequently translated transcripts (6, 9, 226).

Finally, it has recently been documented that posttranscriptional events contribute substantially to *his* operon expression. The native polycistronic *his* message is degraded with a net 5′-to-3′ directionality, generating products that decay at different rates (7, 9, 10).

The different levels of regulation of *his* operon expression will be discussed in more detail in the following sections. Particular emphasis will be given to the contributions of this experimental system to the solution of general aspects of the control of gene expression in prokaryotes.

Regulation of Transcription Initiation

Primary *hisp1* promoter. The *his* operon of *S. typhimurium* and *E. coli* (Fig. 3) is transcribed into a polycistronic mRNA extending from the primary promoter, *hisp1*, to the bidirectional rho-independent terminator located at the end of the gene cluster (52–54, 100, 102, 277).

The primary *hisp1* promoter in *S. typhimurium* and in *E. coli* was initially defined by DNA sequence analysis and by location of the transcription start point (28, 53, 78, 100, 102, 277). By inspection of the nucleotide sequence, it was found to be included among the $E\sigma^{70}$ class of promoters (120, 199). In the two closely related microorganisms, the structure of this genetic element is almost identical, and it is characterized by the presence of three of six matches to the consensus base pairs in both the −10 and −35 hexamers, with a less conserved suboptimal 18-bp separation between the hexamers (Fig. 3). More recently, detailed mutation analysis has provided rigorous evidence that the *hisp1* promoter of *S. typhimurium* is an $E\sigma^{70}$ promoter. All the base pair substitution mutations that decreased its activity altered positions in either the −10 or −35 hexamer (244). Conversely, mutations that improved the match to the consensus sequence of the −10 hexamer increased its intrinsic strength (230). The *hisp1* promoter is strong both in vivo and in vitro, about four times stronger than the *gal* promoter in vivo (225, 277). In vitro, its activity is about 20-fold higher on supercoiled than on linear templates (277). However, it has recently been shown that in vivo treatment of cells with the DNA gyrase inhibitor novobiocin enhanced the expression of a *his-lac* fusion bearing the complete *his* control region and did not substantially modify the expression of a *his-lac* fusion bearing a 35-bp deletion of the *his* attenuator (210). Moreover, repression of *his* expression by anaerobiosis or high osmolarity, two environmental parameters which increase the negative supercoiling of bacterial DNA, required the intact *his* attenuator sequence (210). These findings are consistent with previous observations suggesting that the well-

documented modulation of *his* expression by supercoiling is mostly exerted at the unlinked *hisR* locus, encoding the only $tRNA^{His}$ in the cell (42, 75, 90, 238, 270).

In 1968, Venetianer was the first to propose the existence of positively regulated genes during the stringent response, noting that the *his* mRNA accumulated in the cell following amino acid starvation (275, 276). Until now, most of the information about positive control derives from the *his* operon of *S. typhimurium*. In 1975, Stephens et al. (259) demonstrated that the expression of the *his* operon is subjected to metabolic regulation by showing that the histidine biosynthetic enzyme levels were lower in amino acid-rich than in minimal glucose medium supplemented with histidine. They concluded that the *relA* gene product is required for maximal *his* operon expression in vivo and observed that a strong stimulation of transcription occurred when ppGpp was added to cell-free S30 extracts. The stimulatory effect of ppGpp is believed to be on transcription and not on translation and is promoter specific, as it occurs even when the *his* attenuator is deleted (259, 291).

his operon transcription is maximally stimulated at an intracellular ppGpp concentration below the maximum (259, 291). The most effective concentration of ppGpp is thought to be slightly more than the basal level of this alarmone in minimal-glucose-grown *S. typhimurium* cells. This would account for the slight increase in *his* expression following amino acid downshift in a stringent strain grown in minimal medium. In contrast, attenuator-independent *his* expression in vitro has been shown to vary over a 10- to 20-fold range in correlation with ppGpp levels (259). In an attempt to define a precise correlation between *his* operon expression and ppGpp levels in vivo, Shand et al. (245) developed a mild starvation method by using the serine analog serine hydroxamate, which increases ppGpp levels in a $relA^+$ strain and decreases ppGpp levels in a *relA* mutant. By use of this method, it was possible to show that the full range of regulation of attenuator-independent expression in vivo is 20- to 40-fold and correlates well with the intracellular ppGpp concentration.

Although mechanisms operating at the levels of transcription elongation, mRNA decay, or translation cannot be rigorously excluded (88, 203), several lines of evidence support the conclusion that ppGpp modulates *his* operon expression at the level of transcription initiation. First, the range of stimulation of *hisp1* activity by physiological concentrations of ppGpp in in vitro transcription systems (230, 259) is very close to the overall range of stimulation of *his* operon expression observed in vivo (245). Second, mutations that increased the homology of the −10 hexamer of *hisp1* to the consensus sequence of the $E\sigma^{70}$ promoters or that altered the sequence between the −10 hexamer and the start point dramatically enhanced *his* operon transcription in vitro in the absence of ppGpp and reduced the stimulation of this alarmone to less than a factor of 2 (230). On the basis of these results, Riggs et al. (230) argued that promoters presumed to be positively regulated by ppGpp are partially defective in open complex formation, bearing a suboptimal Pribnow box which does not contain an A residue in the fourth position, as is characteristic of negatively controlled promoters, in addition to differences in the region between the −10 hexamer and the start point (271).

Internal *hisp2* and *hisp3* promoters. In addition to *hisp1*, two weak internal promoters, designated *hisp2* and *hisp3* (Fig. 1 and 3), have been mapped both genetically and physically proximally to the *hisB* and *hisI* cistrons, respectively (26, 82, 113, 243). Although they are quite common in large bacterial operons, the physiological significance of these genetic elements is controversial. Even though it is possible that these promoters are physiologically unimportant and their presence merely fortuitous, their maintenance by selective pressure in homologous genomic regions of related microorganisms (30, 145) supports their physiological relevance. They could reinforce the expression of distal cistrons of large operons, thereby alleviating the effects of natural polarity. Alternatively, they could allow regulation of an operon in a noncoordinate fashion and cause temporally different expression of certain genes under specific growth conditions (see below).

According to several features of the nucleotide sequence, the *hisp2* promoter belongs to the $E\sigma^{70}$ class of promoters. It is subject to metabolic regulation, although to a lesser extent than the primary *hisp1* promoter, its activity being only twofold lower in rich than in minimal glucose medium (113, 291).

The overall contribution of this internal promoter to the expression of the distal genes of the *his* operon is negligible when transcription proceeds from *hisp1* in wild-type cells growing in minimal glucose medium (82). However, its activity increases about threefold under these growth conditions when transcription from *hisp1* in the wild type is abolished, whereas it is almost completely inhibited when transcription from the upstream promoter is very efficient, as in constitutively derepressed mutants (7, 82). Such inhibition of promoter activity by transcription readthrough has been called promoter occlusion and might result from direct steric hindrance of an internal initiation site by RNA polymerase molecules initiating upstream or from distortion of DNA structure (3, 29, 66, 150).

Regulation of Transcription Elongation and Termination

Attenuation control. In both *S. typhimurium* and *E. coli*, expression of the *his* structural genes is coordinately modulated in response to the availability of charged histidyl-tRNA by an attenuation mechanism of transcription at the level of the leader region preceding the first structural gene (24, 28, 78, 151, 156, 162). Since regulation by attenuation has been reviewed in the past more than once, readers are referred to more exhaustive articles for a historical picture of the fundamental steps leading to the discovery of this control mechanism (25, 38, 162).

The purpose of this section is to present the current mechanistic model of attenuation as formulated on the basis of the evidence accumulated to date and to report more recent insights concerning particular aspects of this phenomenon which may be relevant to understanding the fundamental mechanisms governing transcription elongation-termination.

Two prominent features characterize the leader region of the *his* operon which may account for *his*-specific translational control of transcription termination, which is the essence of attenuation control: (i) a short coding region that includes numerous tandem codons specifying histidine (7 histidine codons in a row of 16) and (ii) overlapping regions of dyad symmetry that may fold into alternative secondary structures, one of which includes a rho-independent terminator. In the termination configuration, base pairing involves regions A and B, C and D, and E and F (Fig. 4). The stable stem-loop structure E:F followed by a run of uridylate residues constitutes a strong intrinsic terminator. In the antitermination configuration, base pairing between B and C and between D and E prevents formation of the terminator, thus allowing readthrough transcription. The equilibrium between these alternative configurations is determined by the ribosome occupancy of the leader region, which in turn depends on the availability of charged histidyl-tRNA.

Low levels of the specific charged tRNA will cause ribosomes to stall on the leader region at the histidine codons and to disrupt A:B pairing by masking region A. Under these

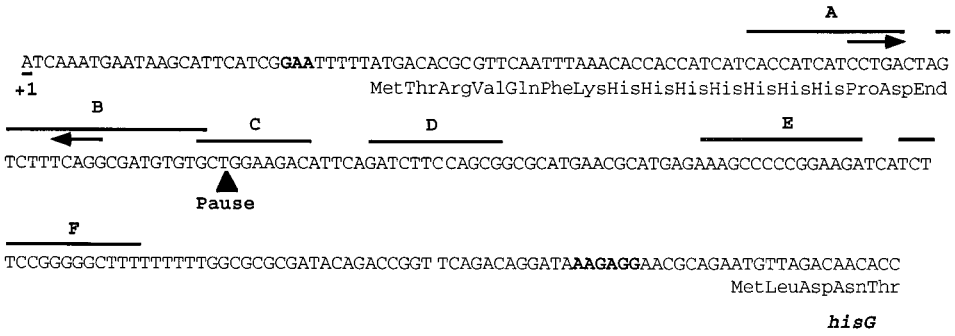


FIG. 4. Features of the leader region of the *his* operon of *S. typhimurium*. The nucleotide sequence of the leader region from the transcription initiation site (+1) to the first structural gene (*hisG*) is reported. Nucleotides corresponding to the Shine-Dalgarno sequences are in boldface. The amino acid sequences of the leader peptide and of the amino-proximal region of the *hisG* gene product are indicated below the nucleotide sequence. Solid lines above the nucleotide sequence correspond to regions (A to F) capable of forming mutually exclusive secondary structures. The convergent arrows indicate the minimal stem-loop structure that is required for transcriptional pausing at a downstream site (solid triangle).

circumstances, the antitermination configuration will be favored. Conversely, in the presence of high levels of charged histidyl-tRNA, ribosomes will rapidly move away from the histidine regulatory codons, thereby occupying both the A and B regions. Pairing between C and D and between E and F will result in premature transcription termination.

Impairment of translation of the leader region as a consequence of severe limitation of the intracellular pool of all charged tRNAs will result in strong transcription termination (superattenuation). Under these conditions, A:B, C:D, and E:F stem-loop structures will form sequentially without interference by actively translating ribosomes.

A characteristic feature common to all known examples of attenuation is a significant pausing by RNA polymerase after synthesis of the first leader transcript RNA hairpin. This pause event is believed to synchronize transcription and translation in the leader region by halting the transcribing RNA polymerase until a ribosome commences synthesis of the leader peptide. Absence of synchronization would not allow complete relief of termination in the presence of low levels of charged histidyl-tRNA (166, 167, 169, 292).

In the *his* leader region, RNA polymerase pausing occurs after synthesis of the first secondary structure (A:B) and immediately prior to the addition of a G residue (57). The position of the pause site allows ribosomes initiating synthesis of the leader peptide to release the paused transcription complex by disrupting the pause hairpin.

Analysis of the effects of base substitutions upstream from the pause site revealed that the pausing signal is multipartite and consists of at least four distinct components: (i) a 5-bp nascent transcript stem-loop structure (the pause hairpin); (ii) the 11-nucleotide 3′-proximal segment of transcript or DNA template; (iii) the 3′-terminal nucleotide; and (iv) the immediate downstream DNA sequence (57, 58, 172, 173). The behavior of compensatory substitutions in the A:B hairpin region suggested that the *his* pause hairpin corresponds to only the upper portion of the larger A:B secondary structure (58). Such a finding is consistent with the deduced structure of RNA and DNA chains in purified transcription complexes paused at the *his* leader region, which has been determined by analyzing the reactivity of specific residues on DNA to chemical modifying agents and the sensitivity of the nascent RNA molecules to RNase A (172). This analysis showed that in spite of considerable variation in the size of the transcription bubble during elongation, the 3′-proximal nucleotides of the transcript constantly pair with the DNA template and that the DNA-RNA hybrid is not disrupted by hairpin formation at the pause site. This finding ruled out the possibility that extensive secondary structures halt elongation by removing the 3′ end of the transcript from the catalytic site of RNA polymerase or by disrupting pairing between the 3′-proximal segments of transcript and DNA template (166, 168). Chan and Landick (58) suggested that pausing is mediated in part by non-sequence-specific, electrostatic interactions between the phosphate backbone of the pause RNA hairpin and a positively charged region on RNA polymerase. NusA elongation factor may enhance pausing by directly contacting the pause hairpin or by increasing its interaction with RNA polymerase (57, 58).

It has recently been proposed that pausing might depend on discontinuous movements (inchworming) of RNA polymerase along the RNA and DNA chains. These discontinuous movements might trigger pausing and termination by interrupting efficient RNA chain elongation (59). These hypotheses have now been addressed experimentally in the *his* system, and it has been shown that transition from monotonic to inchworm-like translocation immediately precedes RNA polymerase pausing in the *his* leader. Both the 3′-proximal region and the downstream DNA sequence influence discontinuous movements. Upon reaching the *his* pause site, inchworming RNA polymerase either escapes by chain extension or isomerizes to a paused conformation by extrusion of the pause hairpin and a ~10-bp forward jump of its downstream DNA contacts (281).

Polarity. In polycistronic operons, certain mutations which cause premature arrest of translation not only affect the gene in which they occur, but also reduce the expression of downstream genes. This phenomenon is commonly called polarity. Although it was first described in the lactose system (98, 148), the coordinate effect of polar mutations on expression of downstream cistrons and the existence of polarity gradients were better defined in the *his* system by using a large collection of polar mutations in a number of different cistrons (16, 42, 92, 135, 152, 186, 187, 224). The nature and the physiological significance of polarity have been controversial for a long time (see the Introduction). The phenomenon has been explained by postulating the existence of intracistronic cryptic rho-dependent terminators (2, 76). According to a general model of transcriptional polarity (2), premature arrest of translation would favor binding of rho to the nascent transcript, interaction of this terminator factor with elongating RNA polymerase, and subsequent release of the transcript.

This model has been supported by more recent studies on polarity in the *his* operon of *S. typhimurium*. Several cryptic

rho-dependent terminators which are activated by uncoupling of transcription and translation have been identified within the *his* operon by analyzing the origin of truncated *his*-specific transcripts produced in vivo in strains harboring polar mutations scattered in four cistrons of the *his* operon and by in vitro transcription studies (6, 8, 9, 64, 65, 231).

A comparative analysis of the intracistronic terminators in the *his* operon allowed the identification of common features of these signals and led to a more complete understanding of the molecular mechanism by which rho causes termination of nascent transcripts. A consensus motif consisting of a cytosine-rich and guanosine-poor region that is located upstream of the heterogeneous 3′ endpoints of the prematurely terminated transcripts has been detected in all rho-dependent terminators analyzed in the *his* operon as well as in other systems (8, 231). This region, the transcription termination element (TTE), is believed to be the binding activation site of rho protein on nascent RNA (31, 190, 293). Deletions which eliminate or reduce the extent of these TTEs impair transcription termination (60, 103) and relieve polarity (64, 65). Release of transcripts occurs downstream to TTEs at multiple sites (termination sites) in coincidence with RNA polymerase pause sites (8, 231).

The occurrence of more than one TTE in a given cistron accounts for the higher degree of polarity generated by promoter-proximal than by distal mutations. Moreover, the variable number and spatial distribution of TTEs within different cistrons explain the differences in the shapes of polarity gradients. The existence of two closely spaced TTEs in the proximal part of *hisG* accounts for the discontinuity of the polar effects (92). The location of a single TTE toward the distal end of *hisA* results in a similar degree of polarity exhibited by all mutations irrespective of their relative position in this cistron (92). The sequential use of more than one TTE results in an additive effect for the more proximal mutations in *hisD* and generates a real gradient in the cistron (92). The bimodal shape of the gradient in *hisC* (186) is due both to the presence of several TTEs tightly clustered in the proximal and distal regions of the cistron and to the occurrence of concomitant processing events (8).

The unusual features of a class of polar and prototrophic mutations which map in the intercistronic *hisD-hisC* region has contributed to understanding of the physiological significance of intracistronic termination (6, 224). In the wild-type *his* operon, the *hisD* and *hisC* cistrons overlap, in that the termination UGA codon of the proximal *hisD* cistron and the start AUG codon of the distal *hisC* cistron share the central UG dinucleotide (AUGA) (53, 229). The mutation *hisD2352*, which maps in this region, is a G-C/T-A transversion, which changes the stop codon UGA of the *hisD* cistron to a sense codon, allowing the ribosome to continue translation until a new stop codon is encountered 30 nucleotides downstream. At the same time, the transversion changes the initiation codon of the *hisC* cistron from AUG to the triplet AUU, which can still be used as an initiation codon, albeit with reduced efficiency. This in turn generates a strong polar phenotype by inducing premature transcription termination in *hisC* in spite of the persistence of residual levels of translation (6). To a certain extent, premature transcription termination has been detected in *hisC* in wild-type strains grown in the presence of antibiotics which are believed to lower the intracellular pool of fMet-$tRNA_f$, thereby impairing efficient translation initiation (9). These results support the proposal that transcriptional polarity does not require complete arrest of translation (258) and that the degree of premature rho-dependent termination is inversely proportional to the efficiency of translation (6, 9, 226). Therefore, the physiological significance of intracistronic termination might be to modulate the levels of transcription in response to the actual rate of protein synthesis.

Posttranscriptional Regulation: mRNA Processing and Decay

The unstable primary 7,300-nucleotide-long transcript of the *his* operon has a half-life of about 3 min in cells growing in minimal glucose medium and is degraded with a 5′-to-3′ directionality. The decay process generates three major processed species, 6,300, 5,000, and 3,900 nucleotides in length (Pr1, Pr2, and Pr3) (Fig. 3), that encompass the last seven, six, and five cistrons, respectively, and have increasing half-lives (5, 6, and 15 min, respectively) (7, 9, 53). The pattern of *his* mRNA decay is identical in *S. typhimurium* and *E. coli* irrespective of the presence of a 102-nucleotide-long REP sequence in the intercistronic *hisG-hisD* region of the former microorganism which is absent in the other (see above and reference 53).

RNase E controls the decay of the native transcript. Active translation of the 5′-end-proximal cistrons of the processed Pr1 and Pr2 species is required to temporarily stabilize these species (5). The overall process of decay may have functional relevance in balancing the expression of the promoter-proximal genes, which are the first to be transcribed, and the distal ones.

The most distal 3,900-nucleotide-long processed species has a half-life of about 15 min. The uncommon stability of this molecule suggests that the processing event that generates it has functional consequences. In fact, the processed species spans the distal cistrons which are involved, in addition to histidine biosynthesis, in a purine recycling pathway leading to production of the cellular alarmone ZTP (see above) (9). Notably, the same distal genes are also transcribed from the internal *hisp2* promoter, which is subject to promoter occlusion (see above).

The specific processing event leading to production of the 3,900-nucleotide species is mechanistically complex. It requires sequential cleavages by two endoribonucleases. RNase E triggers the process by cleavage either at a major target site located in the *hisC* cistron 620 nucleotides upstream of the 5′ end of the processed species (Fig. 3) or at more infrequently used sites (7, 10). RNase P further cleaves the products generated by RNase E at a discrete site located 76 nucleotides upstream of the start codon of the *hisB* cistron, thus generating the mature 5′ end (10). The RNase P-dependent cleavage occurs at the 5′ end of a region that may fold into a short stem-loop structure followed by a 3′-distal NCCA sequence (Fig. 3). RNA molecules with such features have been proposed to be minimal substrates for this endoribonuclease (12, 95, 127). The considerable stability of the processed species may be conferred by the stem-loop structure sequestering the 5′ end of the mature RNA generated by RNase P (40).

Translational events modulate the mRNA processing efficiency. The RNase P-catalyzed reaction requires binding of ribosomes at the ribosome-binding site of the *hisB* cistron (7, 10). Ribosomes initiating translation of *hisB* might favor the formation of the RNase P-targeted structure, allowing RNase P to cleave mRNA efficiently. Alternatively, they might stabilize the processed species by temporarily arresting the 5′-to-3′ wave of decay.

Metabolic perturbation of the translation process caused by limitation in the intracellular pool of initiator tRNA results in an increase in the amount of the processed species in vivo (9). This effect may be due to two mechanisms. Low levels of

initiator tRNA might uncouple transcription and translation, thereby improving the exposure of target sites to RNase E, which triggers the process. Moreover, reduction of the intracellular levels of initiator tRNA might affect processing by altering the kinetics of formation of the initiation complex at the intercistronic regions and causing stalling of ribosomes at the *hisB* ribosome-binding site (219, 220). Ribosome stalling would in turn result in a stabilization of the RNase P-targeted structure. Since translation is likely to control mRNA processing and decay in this as well as in other systems (reviewed in references 4, 33, and 218), the exact links of these distinct cellular processes should be clarified in more details.

REGULATION OF HISTIDINE BIOSYNTHESIS IN OTHER SPECIES

As discussed above, the regulation of *his* operon expression in *E. coli* and *S. typhimurium* has been the subject of intensive studies, and the general mechanisms and molecular details of the process are fairly well established. On the other hand, very few studies in this area have been performed with other prokaryotic cells. In general, it seems that while the biochemical reactions leading to histidine biosynthesis are the same in all organisms, the overall genomic organization, the structure of the *his* genes (see pertinent sections), and the regulatory mechanisms by which the pathway is regulated differ widely among taxonomically unrelated groups.

In the closely related organism *K. pneumoniae*, the overall genomic organization, at least of the proximal and distal regions, appears to be conserved (228, 233). DNA sequence analysis of the regulatory region and promoter expression studies under different metabolic conditions and in regulatory mutants also indicate that the mechanisms controlling histidine biosynthesis are well conserved (232). In the gram-negative nitrogen-fixing bacterium *A. brasilense*, a *his* operon comprising five genes has been cloned and characterized (see Organization of the Histidine Genes), but no information on its regulation is available, although a partial characterization of the transcripts has been performed (83).

Several *his* biosynthetic genes have been cloned and sequenced from organisms belonging to the gram-positive group of eubacteria (134, 256) (see appropriate section), but virtually nothing is known about the regulation of histidine biosynthesis in these species. *S. coelicolor his* gene expression appears to be regulated by intracellular histidine levels (51, 177), and the transcription initiation site of the cluster comprising five *his* genes (176) has been determined (177).

Finally, *his* biosynthetic genes from another unrelated group of prokaryotes, the archaebacteria, have also been identified and characterized (see Organization of the Histidine Genes), but again, studies of the regulation of their expression have not been performed.

For all of the above-mentioned systems except *K. pneumoniae*, the only conclusion that can be drawn is that regulation by attenuation can be excluded, since the 5′-proximal regions of all the genes and gene clusters lack both the required palindromic structures and the histidine-rich leader peptide (77). It is believed that studies in this area, making use of the different *his* systems, might be very important and rewarding in the attempt to unravel other mechanisms by which simple unicellular organisms regulate the expression of biochemical pathways.

The situation is completely different for the organisms belonging to the lower eukaryotes, and in particular for *S. cerevisiae*. In these cells, the mechanisms that regulate histidine and other amino acid biosynthetic pathways are well understood, and the *his* genes have been extensively used as a model to study these regulatory systems. Two different *trans*-acting systems are operational: general amino acid control, which activates the transcription of more than 30 genes in 11 biosynthetic pathways in response to amino acid starvation, and basal-level control, which maintains transcription in the absence of amino acid starvation. Both systems are dependent on transcriptional activators (GCN4, BAS1, and BAS2) and a series of accessory factors, including kinases and phosphatases. A detailed analysis of these mechanisms goes far beyond the scope of this article and the interested readers are referred to several excellent reviews that cover this subject (23, 131, 132).

EVOLUTION OF THE METABOLIC PATHWAY

Synthesis of Histidine in Possible Prebiotic Conditions

Although the characteristics of the first living systems are unknown and can only be surmised, it is generally accepted that life resulted from a process of chemical evolution during which a number of organic molecules were synthesized as a result of nonenzymatic reactions (211, 212). It is likely that histidine and other imidazole-containing compounds were present in the prebiotic environment. Although histidine was not detected among the products of the first successful simulation of prebiotic synthesis of amino acids (193), the nonenzymatic formation of different imidazole derivatives (247) prompted the experimental study of the synthesis of this peculiar amino acid under possible primitive Earth conditions.

The chemical synthesis of histidine was achieved in 1911 by W. Pyman, but neither the conditions nor the precursor molecules employed are relevant from a prebiotic perspective. Following the nonenzymatic formation of imidazole from a mixture of glyoxal, ammonia, and formaldehyde (214), it was shown that imidazole-4-glycol and imidazole-4-acetaldehyde could be synthesized from erythrose and formamidine, both of which can be considered prebiotic reactants (248). Later it was demonstrated that imidazole-4-acetaldehyde, which is a product of the dehydration of the anionic form of imidazole-4-glycol, was present among the reaction products of erythrose and formamidine and can be directly converted to histidine by a Strecker cyanohydrin synthesis involving the hydrolysis of a histidyl nitrile intermediate (249). Also of relevance is the early work by Ames on synthesizing D-erythro-IGP and D-erythro imidazole glycerol from D-ribose phosphate and formamidine or D-ribose and ammonia and formaldehyde in a one-step aqueous synthesis under mild conditions. These studies led to the correct speculation that the pathway started with ribose-5-phosphate (12a, 12b, 18a).

Synthesis of the dipeptide histidyl-histidine under possible primitive conditions, involving the evaporation of an aqueous solution of histidine in the presence of condensing agents, has been achieved and is an efficient process (247). Histidyl-histidine is known to catalyze the formation of peptide bonds and to promote the oligomerization of glycine (286) and can have an enhancing effect in some prebiotic reactions involving nucleotide derivatives and oligonucleotides, such as in the dephosphorylation of deoxyribonucleoside monophosphate, in the hydrolysis of oligoA(12), and in the oligomerization of 2′,3′-cyclic AMP under cyclic wet-dry laboratory reaction conditions simulating a primitive evaporating pond (246). This body of data supports the hypothesis that simple peptides of prebiotic origin containing at least two imidazole groups could act as concerted acid-base catalysts and may have played a significant role in the events preceding the emergence of life itself.

Origin and Early Evolution of the Histidine Biosynthetic Pathway

The catalytic properties of imidazole are well established and probably account for the presence of histidine in the active site of many enzymes. It is generally accepted that its imidazole residue allows histidine (i.e., α-amino-4-imidazole propionile) to function as a general acid-base catalyst by proton relay mechanisms in a number of biochemical reactions (36, 89, 282).

The wide variety of imidazole-containing compounds that can be formed nonenzymatically suggests that a number of homologs of histidine, such as γ-(4′-imidazolyl)-α-amino butyric acid, may have existed in the primitive environment. However, the available evidence suggests that the enhanced stability of histidine and its derivatives, such as its tRNA ester, compared with that of other possible imidazole-containing amino acids may have played a major role in its selection (282). If histidine was required by primitive biological catalysts, then the eventual exhaustion of the prebiotic supply of histidine and histidine-containing peptides must have imposed an important selection pressure favoring those organisms capable of synthesizing imidazole-containing compounds. Although there are several independent indications for the antiquity of the histidine biosynthetic pathway (87, 170), at present the origin of this route is unknown.

Although purines are imidazole-containing compounds which are likely to have been components of primordial biological systems, in RNA they are present as ribosylated derivatives. Thus, the catalytic abilities of their imidazole moieties are suppressed as a result of their linkage to ribose, and imidazole itself appears to play no role in ribozymic activities. However, the ease of the condensation reaction under possible prebiotic conditions of formaldehyde with uracil to give 5-hydroxymethyluracil and the extremely efficient addition of the latter with imidazole suggest that during the RNA world, analogs of histidine may have existed and may have enhanced the catalytic abilities of primitive living systems (231a).

Since the biosynthesis of histidine requires a carbon and a nitrogen equivalent from the purine ring of ATP, it has been proposed that histidine itself may be the molecular descendant of a catalytic ribonucleotide dating from an earlier biochemical stage in which RNA played a major role in biological catalysis (287). However, this suggestion failed to provide a detailed explanation of how the anabolic route was assembled, nor did it explain the origin of the enzymes mediating it. The possibility that histidine biosynthesis evolved backwards from intermediates present in the primitive broth (138, 139) is also unlikely, since it is difficult to envision the prebiotic formation and accumulation of such activated, unstable compounds. At present, it seems likely that a testable description of the emergence of the histidine biosynthetic pathway should invoke spontaneous chemical reactions leading to the formation of the imidazole ring and its condensation with other components of histidine, as well as a coherent description of how primitive, less specific biological catalysts may have taken over the entire sequence of reactions.

Even if we do not know how histidine biosynthesis actually originated, analysis of the extant pathway may be used to compare different and sometimes contradictory theories that have been suggested to account for the establishment of anabolic routes. These explanations include the retrograde hypothesis (138, 139), according to which biosynthetic pathways evolved in a stepwise and backward fashion from the final product of the route. This explanation proposes that the group of genes involved in a particular anabolic pathway are the result of duplication and divergence of a common ancestral gene. Another explanation is the possibility that at least some biosynthetic pathways evolved forward, i.e., from simple precursors to complex end products (111). It has also been suggested that metabolic routes appeared as a result of the gradual accumulation of mutant enzymes with minimal structural changes (280). Finally, according to the patchwork theory, metabolic routes are the result of the serial recruitment of relatively small, inefficient enzymes endowed with broad specificity that could react with a wide range of chemically related substrates (149, 295). This explanation requires the previous existence of ribosome-mediated synthesis of catalytic proteins.

A cladistic analysis of the available *his* genes does show that paralogous gene duplications have played a major role in shaping the pathway. This is indicated by the evidence of two successive duplications involving an ancestral module which eventually led to the *hisA* and *hisF* genes and their homologs (see below). On the basis of this analysis, it has been postulated that the *hisA* and *hisF* genes and their homologs are the descendants of a gene encoding a less specific enzyme (87), evidence supported also by the finding that the HisF protein is able to interact, with reduced affinity, with 5′-ProFAR, the substrate of the HisA enzyme (158). Sheridan and Venkataraghavan (251) have proposed a potential substrate recognition site in HisA and HisF on the basis of a common signature in both proteins which was assumed to be a strand-helix-strand structure that could bind glycerol-phosphate moieties. It is then possible that 5′-PRFAR or 5′-ProFAR could have been the substrate of the ancestral HisA enzyme. These speculations appear to support the so-called patchwork hypothesis (149, 295) and are consistent with the possibility that an ancestral histidine pathway may have evolved from a primitive enzyme that catalyzed two or more similar reactions and whose substrate specificity was refined as a result of later duplication events. The possibility that histidine biosynthesis was originally mediated by less specific enzymes is strongly supported by the common origin of the imidazole glycerol-P synthase moiety, encoded by the enterobacterial *hisH* gene, and other *E. coli* G-type glutamine amidotransferases (GAT), which participate in the biosynthesis of purines, pyrimidines, arginine, tryptophan, and other ancient pathways (87). Although for the time being the lack of sequences of the G-type GAT from the three cell lineages limits a complete evolutionary analysis, the similarity data available suggest that they may be the descendants of an ancient, less specific glutamine amidotransferase which mediated the transfer of the amide group of glutamine to a wide range of substrates.

Evolution of the *his* Genes

The evolutionary comparison of the *his* genes in the three cell lineages clearly indicates that, after the divergence from the last common ancestor, the structure, organization, and order of these genes have undergone major rearrangements (Fig. 2). Although it is not possible from the analysis of the available data to infer the organization of the *his* genes in the last common ancestor (that is, whether these genes were clustered or scattered throughout its genome), nevertheless, the same analysis may help to elucidate the primitive structure of some *his* genes and their evolution. It is worth noticing that some of them have undergone gene duplication and/or gene fusion events. Gene fusion in particular, as well as gene duplication, appears to be one of the most important mechanisms of gene evolution in the histidine biosynthetic pathway. Several fusion events have occurred in the genomes of both bacteria and some eukaryotes, leading to longer genes encoding bi- or

multifunctional enzymes. Although gene fusions can be selected for substrate channeling, they also represent an effective mechanism ensuring the coordinate synthesis of two or more enzymatic activities. This may have special significance among eukaryotes, in which the absence of operons does not allow coordinate regulation by polycistronic mRNAs (74).

Evolution of *hisB*. As described above, in enterobacteria *hisB* codes for a bifunctional enzyme catalyzing the sixth and eighth steps of histidine biosynthesis (289). The most widely accepted model for the association of these two enzymatic activities of the *hisB* gene product envisions the existence of two independent domains in the gene, i.e., a proximal domain encoding the phosphatase moiety and a distal one encoding the dehydratase activity (41, 63, 178). The structural organization of the two enzymatic activities in other microorganisms in which they are encoded by two separate genes confirms the two-domain model discussed above (Fig. 2). Altogether, the available data support the idea that a bifunctional *hisB* gene is an enterobacterial peculiarity, i.e., it seems likely that the evolution of the *hisB* gene in *E. coli* and *S. typhimurium* could have involved the fusion of two independent cistrons, *hisB*px and *hisB*d, coding for a HOL-P phosphatase and an IGP dehydratase, respectively (85, 87). The lack of significant sequence homology between the two moieties of the enterobacterial *hisB* gene suggests that domain shuffling rather than gene duplication or gene elongation is responsible for its present-day structure. Moreover, it is likely that this fusion event took place after the evolutionary splitting between the α and γ branches of the purple bacteria, as *A. brasilense* also showed separate genes.

Evolution of *hisI* and *hisE*. As reported above, the *hisI* and *hisE* genes are fused in *E. coli*, *S. typhimurium*, *L. lactis*, *S. cerevisiae*, *P. pastoris*, and *N. crassa* but separated in *A. brasilense* and *M. vannielii*. Two contrasting hypotheses could explain this difference. In one of them, an ancestral *hisIE* bifunctional gene gave rise in some prokaryotes to monofunctional genes by at least two independent splitting events. Alternatively, two ancestral genes, each encoding a monofunctional enzyme catalyzing sequential steps in histidine biosynthesis, might have undergone many independent fusion events in different cell lineages (87).

In lower eukaryotes, the *hisI* and *hisE* genes are fused to *hisD*, giving a larger multifunctional gene. As shown in Fig. 2, these genes have the same internal organization, with the *hisD* domain located in the 3′-terminal region. It has been suggested that this eukaryotic multifunctional gene originated from the fusion of bacterial separated cistrons (45), but just how many gene fusion and/or gene elongation events led to the extant fungal genes is an open question. Moreover, since the *HIS4* and *his-3* genes have the same internal organization, the putative fusion event(s) took place before the evolutionary separation of these fungi but after the separation of fungi from plants. In fact, the *hisD* gene of *B. oleracea* is not fused to the *hisI* and *hisE* genes, demonstrating that the evolution of *his* genes followed different paths among eukaryotes.

Evolution of paralogous *his* genes. An additional intriguing feature of histidine biosynthetic genes concerns the two genes *hisA* and *hisF*. The analysis of the sequence of the *hisF* gene and of its product demonstrated a remarkable homology with *hisA*, the nearest preceding gene in eubacterial operons, and with its product, 5′-ProFAR isomerase (86). This homology, recognized in all *hisA* and *hisF* genes sequenced, has been interpreted as the consequence of a gene duplication event during the evolution of the operon (see below). The same analysis, moreover, demonstrated the presence of two homologous moieties inside each of the two genes *hisA* and *hisF* which appear to be composed of four relatively homologous modules, A1, A2, F1, and F2, which are the result of two successive duplication events, the first one involving the *hisA*1 module and leading to the extant *hisA* gene, which in turn duplicated and gave rise to the *hisF* gene (86).

In *S. cerevisiae*, the *hisF* counterpart is represented by *HIS7*, which is composed of two moieties corresponding to the *E. coli hisH* and *hisF* genes (164). Analysis of the deduced amino acid sequence of the HisF moiety coded by the 3′ region of *HIS7* revealed a high degree of sequence similarity with the prokaryotic HisA proteins, especially with the archaebacterial ones. This moiety, like its bacterial counterpart, is formed by two homologous modules half the size of the entire moiety (87). More recently, the *HIS6* gene of *S. cerevisiae*, homologous to the prokaryotic *hisA*, has been cloned and analyzed (84). Its product shows a significant degree of sequence homology with the prokaryotic HisA proteins, with the known eubacterial HisF proteins, and also with the 3′ domain of yeast *HIS7* (84), indicating that the *S. cerevisiae HIS6* and *HIS7* genes are also paralogous. The *HIS6* gene also shows the two-module structure typical of all the known *hisA* and *hisF* genes. This set of data suggests that *hisA* (and probably *hisF*, although an archaebacterial *hisF* gene has not been identified yet) was part of the genome of the last common ancestor and that the two successive duplication events leading to the extant *hisA* and *hisF* took place long before the diversification of the three evolutionary domains, probably in the early stages of the molecular evolution of the histidine pathway.

The evolutionary history of *hisH* also probably involves a duplication event. In fact, the sequence similarity of the different imidazole glycerol P synthase moieties encoded by the enterobacterial *hisH* genes with the sequences encoding the so-called G-type GAT genes encoding the anthranilate synthase (208), 4-amino-4-deoxychorismate synthase (155), carbamoyl-P synthase (222), GMP synthase (269), CTP synthetase (285), and formylglycinamide synthetase (241, 242) implies that these different enzymes are also the products of ancient paralogous duplications (87). From an evolutionary point of view, particularly interesting is the *S. cerevisiae HIS7* gene, which appears to be constituted by the two bacterium-like cistrons *hisH* and *hisF* (164), which in turn are the results of two series of independent gene duplication events. As previously reported, the *E. coli hisF* and *hisH* genes code for proteins which associate to form a heterodimeric enzyme. The genes encoding them are not adjacent in any of the bacteria studied but are fused in *S. cerevisiae* as a result not of an elongation event but of a domain-shuffling phenomenon (87). However, the possibility that the *HIS7* gene originated from the fusion of two genes via the deletion of the intervening region cannot be ruled out. As noted before, the 3′-terminal region of *HIS7* is considerably longer than that of the eubacterial *hisF* genes because of the presence of six insertions in the first module (*hisF*1). Since these insertions are not present in *HIS6*, it is likely that they were incorporated in *HIS7* after the divergence of prokaryotes and eukaryotes. Nevertheless, we cannot a priori rule out the possibility that these insertions were incorporated in an ancestral *HIS7* gene before this divergence. The cloning and analysis of archaebacterial and additional eukaryotic *hisF* genes might solve this issue.

Evolution of *his* clusters. Differences in the relative *his* gene order may be observed in those prokaryotes in which at least some of the histidine biosynthetic genes are clustered, as reported before. Nevertheless, four of the clustered genes (*hisB*d, *hisH*, *hisA*, and also *hisF* except in *S. coelicolor*) are always present and in the same relative order. These four genes encode enzymes involved in the central, sequential en-

zymatic steps of the pathway, connecting histidine biosynthesis with nitrogen metabolism and the de novo synthesis of purines (see above).

It is possible that the four genes *hisB*d*HAF* represent the core of histidine biosynthesis and that the *his* operon is an open plastic operon, i.e., an operon in which, except for the highly maintained core of the pathway, different gene organizations are possible (87). Indeed, the known *his* operons show different gene organizations, and most of them (*A. brasilense*, *S. coelicolor*, and *L. lactis*) also contain nonhomologous ORFs with unknown function. Moreover, it is possible that this set of enzymes (or at least HisF and HisH proteins) are a metabolon, as defined by Srere (257), promoting the preferential transfer of an intermediate metabolite from one enzyme to a physically adjacent enzyme and limiting its diffusion into the surrounding milieu (189). The question of whether these four genes could have acted as a unitary block in the evolution of the eubacterial *his* genes will be solved when additional *his* operons from other different eubacteria are described in detail.

Molecular Phylogenies of the *his* Genes

Because of the availability of so much sequence data for so many different organisms, the *his* biosynthetic genes constitute a precious opportunity to perform phylogenetic studies. The molecular phylogenetic analysis of the *his* genes, performed in order to compare the evolutionary relationships among the organisms from which the different genes involved in the pathway have been sequenced, indicated the existence of three cellular domains (87). Some peculiarities of *his* gene evolution are worth mentioning. The detailed analysis of the phylogenetic trees showed that the nitrogen-fixing α-purple gram-negative bacterium *A. brasilense* was in most cases nearer to gram-positive bacteria than to its close relatives, the γ-purple enterobacteria *E. coli* and *S. typhimurium*. The same result was found for *K. pneumoniae* when the first 100 amino acids of its *hisG* gene product were compared, but the latter result was not confirmed by the analysis of the HisF proteins, which placed *K. pneumoniae* closer to *E. coli* and *S. typhimurium*. Whether the peculiar position of these two nitrogen-fixing eubacteria reflects an ancient lateral gene transfer event is not clear yet. Another interesting feature of *his* gene evolution concerns the trees depicting the phylogenies of the *hisC* and *hisI* gene products, which point towards the evolutionary proximity of the low-G+C gram-positive branch (*B. subtilis* and *L. lactis*) to the Archaea rather than to the other bacteria (87). This observation is consistent with results obtained by the comparison of glutamate dehydrogenase (35), heat shock proteins (117, 118), glutamine synthetase (44, 165, 268), and carbamoyl synthetases (171), all of which have been interpreted as indicating an early massive lateral gene transfer event between the ancestors of both Archaea and gram-positive bacteria (106).

CONCLUSIONS

The histidine biosynthetic pathway is unique in the presence of several reactions quite unusual for a biosynthetic pathway, in the existence of links with other metabolic pathways, and in the structural features of several of the biosynthetic enzymes. In *S. typhimurium* and *E. coli*, seven enzymes coded for by eight genes control 10 enzymatic steps: three of them are bifunctional, and one is heterodimeric, being composed of the *hisH* and *hisF* gene products.

Regulation of the activity of the first enzyme of the pathway by the end product histidine and by several other molecules signaling the energy charge of the cell represents a sophisticated example of enzymatic feedback inhibition. The study of the correlation between the aggregation state of the enzyme and its activity will help to clarify the mechanism of allosteric inhibition.

Comparison of the structural properties of several *his* gene products, each catalyzing more than one biosynthetic step, provides an opportunity to study the evolution and the mechanisms of catalysis of bifunctional and multifunctional enzymes. HOL-dehydrogenase, which completes the biosynthetic pathways by oxidizing the amino alcohol HOL to the corresponding amino acid histidine through the unstable amino aldehyde HAL, represents one of the first examples of bifunctional NAD^+-linked dehydrogenases. Recent studies have suggested that the route for the aldehyde oxidation might proceed through a scheme different from that common to most aldehyde dehydrogenases. Definition of the precise mechanism by which this reaction is carried out will provide a new paradigm for aldehyde oxidation.

The last blind spot in the histidine biosynthetic pathway, the conversion of 5-PRFAR to IGP and AICAR, has been elucidated only recently. The finding that in enterobacteria the *hisH* and *hisF* gene products, in analogy to other glutamine amidotransferases, associate to form the dimeric active IGP synthase holoenzyme catalyzing this step will help to elucidate the mechanisms of catalysis and the functional interactions of monomers in multimeric enzymes.

The purine and histidine biosynthetic pathways are connected through the AICAR cycle. The AICAR is converted either to IMP, in the presence of adequate folate levels, or to the unusual nucleotide ZTP, under metabolic conditions inducing folate starvation. ZTP is a proposed alarmone signaling C-1-folate deficiency and mediating a physiological response to folate stress. Although inhibition of folate metabolism actually leads to alterations in a number of intracellular processes by inducing metabolic transcriptional polarity and affecting mRNA decay and processing or by triggering other phenomena, such as sporulation in *B. subtilis* and the process of nodule formation by *Rhizobium* species, the involvement of ZTP in these processes is only speculative, and further studies will be required to demonstrate the existence of a folate stress regulon.

Overproduction of AICAR has been previously invoked to account for the multiple phenotypic changes resulting from the constitutive expression of the *hisH* and *hisF* gene products. However, the evidence that the pleiotropic response still occurs in strains devoid of AICAR as a consequence of interruption of the carbon flow through the histidine and purine pathways has ruled out this hypothesis. Intergenic suppression analysis has led to the speculation that several aspects of the pleiotropic response (e.g., filamentation) might be due to the interference of elevated expression of *hisH* and *hisF* with the synthesis of the cell wall. This genetic approach allowed the identification of novel loci on the *S. typhimurium* chromosome which control synthesis of cell wall and the establishment of a hierarchy between them by observing epistatic effects among suppressors.

The investigation of the mechanisms regulating the expression of the *his* operon in *S. typhimurium* and *E. coli* has helped to clarify general aspects of the control of gene expression in prokaryotes: control of transcription initiation, attenuation, polarity, mRNA processing, and decay.

Although transcription attenuation was demonstrated to regulate *his* expression and was formally theorized in its essence more than 15 years ago, several aspects of the mechanism regulating the coupling of transcription and translation at a transcriptional pausing site within the leader region have

been elucidated only recently. Understanding of several mechanistic features of transcription attenuation in the *his* leader has helped to demonstrate that discontinuous movements of RNA polymerase are steps in the pausing and termination processes.

The availability of a large collection of polar mutations scattered along the different cistrons of the operon has provided the opportunity to study the mechanisms responsible for polarity. These studies have led to the formulation of a mechanistic model of activation of rho termination factor at the level of intracistronic terminators and to the envisaging of the existence of a general mechanism regulating transcription in response to the rate of protein synthesis, operating at the level of these terminators. The structure of the transcription elongation complex, the nature of the *cis* signals on RNA and DNA modulating its activity, and the role of ribosomes in controlling the elongation step of transcription are relevant subjects in this field of research.

Posttranscriptional mechanisms have more recently been shown to regulate the expression of the *his* operon. A relatively stable transcript spanning the distal *hisBHAFI* cistrons is originated by sequential cleavage by RNase E and RNase P. The relevance of the processing event in the cellular economy is not clear. The presence of an internal promoter, evolutionarily conserved between *E. coli* and *S. typhimurium* and driving transcription of the same distal cistrons contained in the processed species, suggests the existence of a selective pressure favoring the differential expression of these cistrons, whose products are also involved in metabolic pathways other than histidine biosynthesis. The study of the processing reaction, which represents the first example of cleavage of an mRNA by RNase P, will contribute to understanding how this ribozyme is targeted to substrates other than tRNA precursors.

Another area which might be exploited for the understanding of important biological functions is the study of mechanisms governing the regulation of expression of the histidine pathway in different organisms. In fact, while so many genes and clusters have been characterized in a number of different species, studies of the mechanisms by which their expression is controlled have not been performed yet. Attenuation seems to be specific to the enterobacteria and the general amino acid and basal-level controls to yeasts. Each organism seems to have evolved and adapted different ways of controlling gene expression, and studies with the *his* genes as a model system could be very rewarding.

The availability of information about more than 60 *his* genes from a wide range of microorganisms belonging to the three cell domains has permitted a deep analysis of the structure, organization, and evolution of the *his* biosynthetic genes. As reported above, there are many clues indicating the antiquity of this anabolic pathway, suggesting that the entire pathway might have been assembled well before the appearance of the last common ancestor cell.

The synthesis of histidine and of the dipeptide with catalytic activity histidyl-histidine under possible prebiotic conditions suggests that these molecules were present in the primordial soup and that the pathway might have assembled as a consequence of the exhaustion of the prebiotic supply of histidine and histidine-containing peptides. These events must have imposed an important pressure favoring those organisms capable of synthesizing imidazole-containing compounds. The analysis and the comparison of the structure of some of the *his* genes (*hisH*, *hisA*, and *hisF*) suggests that paralogous gene duplications have played an important role in shaping the pathway. It has also been postulated that the products of these three genes and their homologs are the descendants of genes encoding less specific enzymes, supporting the so-called patchwork hypothesis (149, 295).

After the building of the entire pathway, these genes underwent several major rearrangements in the different cellular lineages. In the eubacteria, some of the *his* genes are clustered in an operon. This is particularly true for *hisB*d, *hisH*, *hisA*, and often *hisF*, which have always been found contiguous and arranged in the same order. This group of genes might have acted as a unitary block in the evolution of the eubacterial pathway.

Although limited information about the organization of the *his* genes in Archaea exists, the available data indicate that they are scattered on the chromosome, resembling the situation in lower eukaryotes.

The phylogenetic comparison of the available *his* gene sequences is consistent with the existence of three cellular domains, but this analysis also placed the nitrogen-fixing α-purple gram-negative bacterium *A. brasilense* and some Archaea near the low-G+C gram-positive bacteria, including *B. subtilis* and *L. lactis*, which probably reflects massive lateral gene transfer events. Therefore, the *his* genes and their products can be used to establish the phylogenetic relationships among microorganisms and may also evidence events of lateral gene transfer. Moreover, since paralogous genes are often used for rooting the phylogenetic trees, a systematic study of the *his* paralogous duplications that took place in the early stages of molecular evolution may provide promising sets of data for the construction of deep phylogenies that might shed light on the proper rooting of the universal trees.

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ADDENDUM IN PROOF

Since submission of this review, the formidable achievement of sequencing the first complete genome of a living organism, *Haemophilus influenzae* Rd, has been published. (R. D. Fleischmann, M. D. Adams, O. White, R. A. Clayton, E. F. Kirkness, A. R. Kerlavage, C. J. Bult, J.-F. Tomb, B. A. Dougherty, J. M. Merrick, K. McKenney, G. Sutton, W. FitzHug, C. A. Fields, J. D. Gocayne, J. Scott, R. Shirley, L.-I. Liu, A. Glodek, J. M. Kelley, J. F. Weidman, C. A. Phillips, T. Spriggs, E. Hedblom, M. D. Cotton, T. R. Utterback, M. C. Hanna, D. T. Nguyen, D. M. Saudek, R. C. Brandon, L. D. Fine, J. L. Fritchman, J. L. Fuhrmann, N. S. M. Geoghagen, C. L. Gnehm, L. A. McDonald, K. V. Small, C. M. Fraser, H. O. Smith, and J. C. Venter, Science **269:** 496–512, 1995). The chromosome of this gram-negative eubacterium contains a *his* operon (accession numbers U32730 and L42023) similar to those found in the closely related *E. coli* and *S. typhimurium* species. The order of the genes in the operon is conserved, but curiously, the individual cistrons do not overlap extensively and, except for *hisA*/*hisF* and *hisF*/*hisI*, are separated by 35/111-bp-long intercistronic regions. The features of the 5′ region of the operon (nucleotides 64 to 221) are compatible with a mechanism of transcriptional regulation achieved by attenuation: (i) a 12-amino-acid-long leader peptide with four contiguous His res-

idues or (ii) the presence of mutually exclusive alternative secondary structures allowing, in the absence of or with efficient translation, or preventing, when ribosomes are stalled on the leader peptide region, the formation of a rho-independent termination structure.

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