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Genetics and Molecular Biology of Siderophore-Mediated Iron Transport in Bacteria

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"It is better to wear out than to rust out."
Richard Cumberland (quoted in Boswell's *Tour of the Hebrides*)

INTRODUCTION

Iron is the fourth most abundant element in the Earth's crust; however, under aerobic conditions and at almost neutral pH it is present as a component of insoluble minerals (26, 68–70, 88, 93). Similar types of constraints are imposed on this metal in biological systems. Thus, in vertebrates, iron is tightly bound by high-affinity iron-binding proteins, such as transferrin and lactoferrin in serum and secretions (7, 14–17). Bacterial growth, in turn, depends on the availability of iron, an essential nutrient that participates in many biological processes, including electron transport chains, and is a cofactor of enzymes of intermediary metabolism (68). Therefore, the possession of specialized iron transport systems may be crucial for bacteria to override the iron limitation imposed by the host or the environment (14, 26, 40, 41, 97).

One of the most commonly found strategies evolved by microorganisms is the production of siderophores, low-molecular-weight iron chelators that have very high constants of association for their complexes with iron (14, 26, 62, 68, 70, 85). Thus, siderophores act as extracellular solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation. Transport of iron into the cell cytosol is mediated by specific membrane receptor and transport systems which recognize the iron-siderophore complexes (3, 4, 26).

It is clear from the above paragraphs that siderophore-mediated utilization by microorganisms of the host vertebrate iron, which is mostly bound by high-affinity iron-binding proteins, becomes an important virulence factor in the establishment of an infection (97). Of course, production of siderophores is not the only mechanism whereby bacteria can utilize the otherwise unavailable iron. Certain pathogenic bacteria, such as *Neisseria gonorrhoeae* and *N. meningitidis* have evolved other approaches to access the iron bound by either transferrin or lactoferrin; these microorganisms possess outer membrane protein receptors that actually recognize the complex of lactoferrin or transferrin with iron, allowing for the internalization of this essential element without the agency of a siderophore (63).

Recently, Zimmermann et al. (117) reported the presence of a mechanistically novel ferric iron transport system in *Serratia marcescens*. Iron assimilation by this system required neither a siderophore nor a receptor protein. Furthermore, iron uptake mediated by this system was independent of the TonB and ExbB functions, which are essential for all other iron transport systems in *Escherichia coli*. A clone harboring an *S. marcescens* deoxyribonucleic acid (DNA) fragment of 4.8 kilobases (kb) was sufficient to express the system in *E. coli*. Polypeptides encoded by this region were identified in *E. coli* transcription-translation systems. The cloned system required chromosomally encoded functions of *E. coli* for the uptake of iron (117).

In this review I will emphasize the siderophore-mediated iron transport system encoded by the pJM1 plasmid in *Vibrio anguillarum* and the enterobactin system found in enteric bacteria. However, I will also include important aspects of other siderophore-mediated systems that had been associated with bacterial pathogenicity, such as the aerobactin system of enteric bacteria, for which a considerable body of information has been published in the past few years; the pyochelin and pyoverdin iron assimilation systems of *Pseudomonas* spp.; and the amonabactin system of *Aeromonas hydrophila*. I will end this review by discussing the potential role of siderophore-mediated systems as virulence determinants in the specific host-bacteria interactions leading to disease.

ANGUIBACTIN-MEDIATED PLASMID-ENCODED IRON UPTAKE SYSTEM OF *V. ANGUILLARUM*

The *V. anguillarum* anguibactin-mediated plasmid-encoded iron uptake system is an important component of the virulence repertoire of this marine fish pathogen (25, 26, 50). My laboratory has now demonstrated that expression of this system requires a stretch of about 25 kilobase pairs (kbp) of the 65-kbp pJM1 plasmid DNA (101, 102). The presence of

the pJM1 plasmid is required for virulence, and our cloning experiments have demonstrated that the portion of this plasmid that plays a role in the virulence phenotype is the iron uptake region. Figure 1 shows a schematic representation of the pJM1 iron uptake region. This segment has interspersed genes involved in the biosynthesis of the siderophore anguibactin as well as components intervening in the actual iron transport process (2, 3, 102). Transposition mutagenesis led to the discovery that these genes are arranged in several transcriptional units. As discussed below, we have also demonstrated the existence of positive and negative regulatory factors that control the expression of the biosynthetic genes for anguibactin as well as the iron transport genetic determinants (3, 101). Negative control is achieved at the level of transcription and is coregulated by the iron status of the cell.

In the next few paragraphs I will first briefly refer to our past work with this system, emphasizing its importance as a virulence factor. I will then turn to an analysis of our latest findings concerning the identification and structure determination of the siderophore anguibactin as well as the complex regulatory circuitry, combining positive and negative controlling elements, resulting in the expression of the pJM1-mediated iron uptake system of *V. anguillarum*.

V. anguillarum causes a terminal hemorrhagic septicemia in salmonid fishes (50, 83). Many isolates of this bacterium possess a plasmid-mediated iron uptake system that is strongly correlated with virulence (25, 28, 104). Strains harboring the 65-kbp plasmid pJM1 are able to grow in iron-limited media containing a variety of iron chelators. However, strains cured of this plasmid could not grow under such iron-limited conditions and were no longer virulent (25). During growth under iron-limited conditions, a system is induced in strains containing the pJM1 plasmid which results in the energy-dependent uptake of iron by the *V. anguillarum* cells (27). This system includes a water-soluble siderophore, anguibactin, which accumulates in the culture medium, and an 86-kilodalton (kDa) outer membrane protein, pOM2, whose presence is correlated with the acceptance and transport of iron into the cell cytosol (2, 26, 101). The pOM2 protein is missing from strains which are unable to grow in media in which the iron is complexed by nonassimilable iron chelators, even when supplied with extra amounts of anguibactin purified from wild-type cells. Mutant strains in which this protein is missing and/or in which anguibactin biosynthesis is impaired have been obtained by transpositional mutagenesis resulting in modified pJM1 plasmids (109). Mutant 775:Tn*1–5*, containing the pJM1 derivative pJHC-91 in which transposon Tn*1* was inserted in genetic unit I, can grow in vitro in iron-limited media only if supernatant from strains containing the wild-type plasmid, and thus plenty of the siderophore anguibactin, is supplied. Therefore, strains harboring pJHC-91 must be able to transport and incorporate iron from anguibactin but are not able to produce this siderophore. Strains containing this plasmid show biosynthesis of the pOM2 outer membrane protein. Another mutant, 775:Tn*1–6*, harboring plasmid pJHC9-8, lacks the ability to synthesize anguibactin as well as the ability to use it when it is supplied from external sources. Plasmid pJHC9-8 is a derivative of pJM1 that resulted from Tn*1* insertion and deletion of most of the iron uptake region. Strains harboring pJHC9-8 not only lack anguibactin production but also do not synthesize the pOM2 protein. It was of interest that experimental infections of salmonid fishes with mixtures consisting of the wild-type strain and the siderophore-deficient, receptor-proficient mutant 775::Tn*1–5* resulted in recovery of both the wild-type strain and the mutant strain, whereas infections with mixtures consisting of the wild-type strain and the siderophore-deficient, receptor-deficient mutant 775::Tn*1–6* resulted in recovery of only the wild-type strain (116). These results demonstrated that anguibactin, the *V. anguillarum* plasmid-mediated siderophore, is produced in vivo in a diffusible form. The level of siderophore in the blood and kidneys was sufficient to provide iron for considerable growth of the avirulent strain lacking the ability to produce the siderophore but possessing the transport functions. These facts emphasize the importance of anguibactin and the iron assimilation system as factors of virulence in *V. anguillarum* (55, 116).

Structure of Anguibactin

Siderophores exhibit a great diversity of structures; however, most of the known siderophores are classified as either phenolates or hydroxamates. Figure 2 shows enterobactin as a typical phenolate and aerobactin as an example of a hydroxamate. We have recently isolated the siderophore anguibactin from the culture medium of iron-starved *V. anguillarum* 775 and were able to characterize it (1) and determine its structure (53), which is also shown in Fig. 2. Anguibactin has a molecular weight of 348, belongs to the phenolate category of siderophores, and is, in fact, a catechol rather than a monophenol. However, despite this classification, its molecular composition is rather unusual. It belongs to a unique structural class, although it shows a certain resemblance to pyochelin (Fig. 2). Anguibactin has been identified as ω-*N*-hydroxy-ω-[[2′-(2″,3″-dihydroxyphenyl)thiazolin-4′-yl]-carboxy]histamine by crystal X-ray diffraction studies of its anhydro derivative, proton and ^{13}C nuclear magnetic resonance spectroscopy of its deferri and Ga(III) complex, fast-atom bombardment (FAB) mass spectrometry, and chemical degradation (53). Therefore, the anguibactin molecule contains catecholate and hydroxamate structures. Single-crystal structure determination of the Ga(III) complex (used instead of iron) of racemized anguibactin showed a 1:1 metal-to-ligand stoichiometry in which the O-hydroxy group, the nitrogen of the thiazolin ring, the hydroxamate (N-O group), and the deprotonated nitrogen of the imidazole ring coordinate the metal ion (53). Our previous mass spectrometry analysis also resulted in a molecular ion characteristic of a 1:1 complex of ferric anguibactin (1).

To characterize the pJM1 DNA regions associated with anguibactin biosynthesis, we have recently examined the effect of insertion mutations, with transposon Tn*3*-HoHo1, in the cloned iron uptake region of pJM1 (101). The insertion mutants defined six genetic units. Mutations in genetic units I, III, V, and VI affected anguibactin biosynthesis only, whereas mutations in genetic unit II affected both anguibactin biosynthesis and iron transport (Fig. 1). Genetic region II will be discussed thoroughly below. Since transposon Tn*3*-HoHo1 contains a promoterless *lacZ* gene, the direction of transcription of the genes where it had inserted could be assessed by assaying the β-galactosidase activity of cells harboring the transposon mutants. Significant levels of β-galactosidase would be the result of transcriptional fusions with the pJM1 iron uptake gene where it inserted. Expression could occur only if the reading frame of the fused *lacZ* was in the same orientation as the direction of transcription of the pJM1 iron uptake gene. Most of the insertions in the anguibactin biosynthetic units resulted in the production of β-galactosidase that was under the control of the iron status of the cell; thus, these regions included genes whose expres-

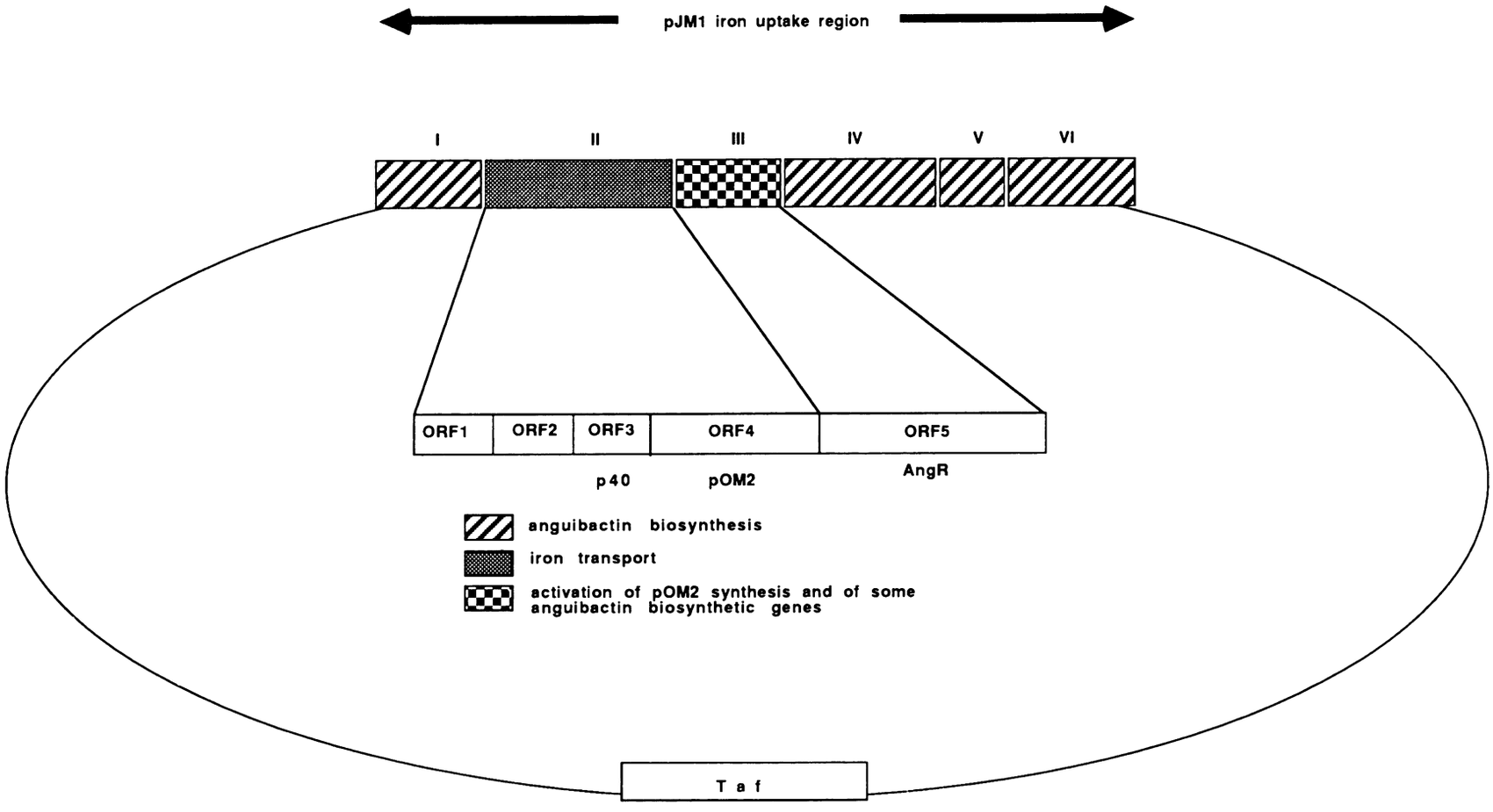

FIG. 1. Organization of the pJM1 iron uptake region. Genetic units I to VI were defined by transposition mutagenesis as described in the text. Insertions in genetics units I, III, IV, V, and VI result in iron-uptake-deficient mutants that do not produce anguibactin but that are proficient in iron transport if anguibactin is provided. Genetic unit II contains the genetic determinants for the actual transport of iron into the cell cytosol. Sequencing analysis identified four ORFs in this region. ORF4 encodes pOM2, an 86-kDa outer membrane protein, and ORF3 encodes the 40-kDa polypeptide p40. These two polypeptides are essential for iron transport. The transcriptional regulators AngR, encoded within genetic unit III, and Taf, encoded in a region noncontiguous to the iron uptake genes, are necessary for the full expression of the siderophore biosynthetic genes as well as for the genetic determinants for iron transport.

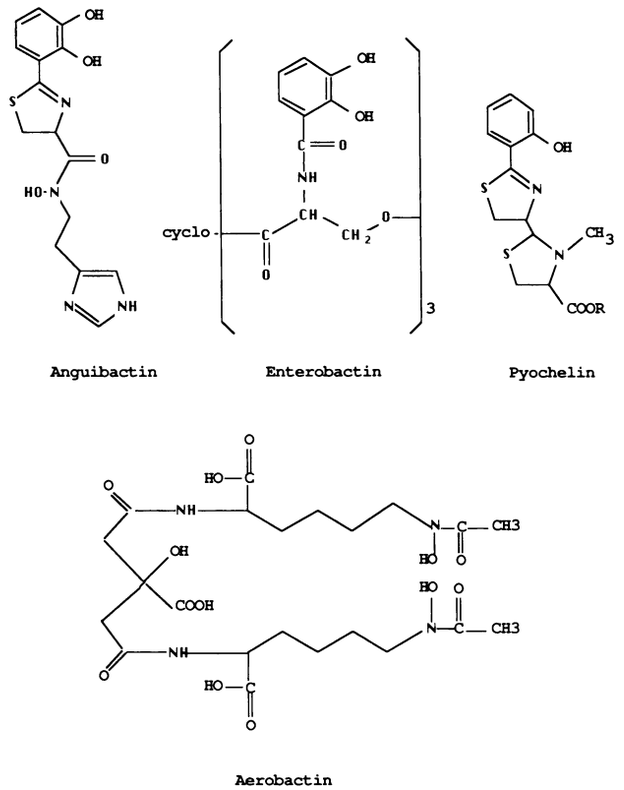

FIG. 2. Structures of the siderophores anguibactin, enterobactin, pyochelin, and aerobactin.

sion was induced under conditions of iron limitation. However, it was of interest that within region I there were overlapping iron-regulated and constitutive transcriptional units that were transcribed in opposite orientations. Work is now under way to identify the actual genes included in these loosely defined genetic regions and their products by complementation of the mutations with clones spanning the mutation site and by physical analysis of biosynthesis intermediates.

trans-Acting Factors and Regulation of the Anguibactin System

A remarkable finding was the requirement of a *trans*-acting factor, designated Taf, encoded by a pJM1 region other than the iron uptake sequences, in order to have full expression of the pJM1-encoded iron uptake system (101, 102). The analysis of anguibactin production as well as β-galactosidase activity demonstrated that Taf must be a transcriptional activator for siderophore biosynthetic genes. However, recent experimental evidence suggests that Taf may also activate the iron transport genes (Salinas et al., manuscript in preparation). Therefore, Taf might be a global positive activator of the pJM1-mediated iron uptake regulon (Fig. 3). The molecular nature of Taf, as well as its site of action on the pJM1 iron uptake genes, is still under study. However, we have to keep in mind that expression of the whole system is negatively controlled by the iron concentration of the cell, possibly by means of a regulatory product such as Fur, the iron-dependent negative regulator of iron uptake systems in *E. coli* (44). The existence of two global regulators, Taf (a positive *trans*-acting factor that is essential under conditions of iron limitation) and the putative iron-associated negative repressor, already suggests a complex regulatory circuitry. Nonetheless, *V. anguillarum* had some more surprises in reserve. This became evident when we dissected genetic unit II in detail.

Mutations in this DNA region resulted in deficiencies in both iron transport and anguibactin biosynthesis (2). This region is contained within an *Eco*RI fragment which harbors the gene for the 86-kDa OM2 outer membrane protein. We had already demonstrated that OM2 plays an important role in the transport of iron into the cell cytosol. To characterize this gene as well as the rest of genetic unit II, we cloned, mutagenized, and sequenced this *Eco*RI fragment (2, 3). The analysis of the nucleotide sequence of this fragment identified five open reading frames (ORFs), all transcribed in the same direction (Fig. 1). The gene encoding OM2 maps within ORF4. An RNA transcript of 2.8 kb, synthesized only under conditions of iron limitation, was identified as the OM2 mRNA. It was of interest that adjacent to the translation termination codon of OM2 there is a region of dyad symmetry reminiscent of a ρ-dependent transcription termination site. The predicted amino acid sequence, as well as the hydropathy plot of ORF4, demonstrated the presence of two hydrophobic regions, one at the N-terminal end and the other in the C-terminal region. The N-terminal hydrophobic sequence corresponded to the signal peptide, whereas the C-terminal end is possibly the anchorage site of OM2 into the bacterial outer membrane. A strongly hydrophilic region in the central region of the protein, spanning about 42 amino acids, may represent a region of OM2 that is exposed to the extracellular medium and is capable of interacting with the ferri-anguibactin complexes.

Mutagenesis analysis identified another region that was essential for iron transport, which was located immediately upstream of the OM2 gene. This region contained ORF1, ORF2, and ORF3; ORF3 was identified as the gene for a 40-kDa polypeptide, p40 (3). The hydropathy plot deduced from the nucleotide sequence of ORF3 suggested that p40 was also a membrane-associated protein. Insertions in this gene not only affected the expression of the p40 protein but also resulted in an iron-transport-deficient phenotype. Thus, p40 must be important, together with pOM2, in the process of recognition of ferric-anguibactin complexes and the subsequent transport of iron into the cell cytosol. The insertion inactivation analysis revealed that certain transposition mutants mapping within ORF1 and ORF2 were also iron transport deficient. pOM2 production in these mutants was rather low, suggesting that in addition to iron transport, these mutations affected the biosynthesis of OM2 either directly or through polar effects of the transposon insertions. Therefore, it appears that the region upstream of OM2 that includes ORF1, ORF2, and ORF3 could play a role in iron transport, as well as being essential for OM2 biosynthesis. However, we have recently found that a region including ORF3 has, in addition to its transport function, an inhibitory action on OM2 biosynthesis (Salinas, Waldbeser, and Crasa, manuscript in preparation). Therefore, this region may encode a product(s) that negatively regulates OM2 synthesis, possibly in conjunction with iron and the transcriptional activator Taf (Fig. 3). Alternatively, this region may contain binding sites for a positive regulator of OM2 synthesis.

In a recent survey of *V. anguillarum* strains isolated from different parts of the world, we came upon another interesting feature which led us to identify yet another regulatory gene, *angR* (87a). Analysis of the iron uptake system from these strains indicated that in all cases it was encoded in

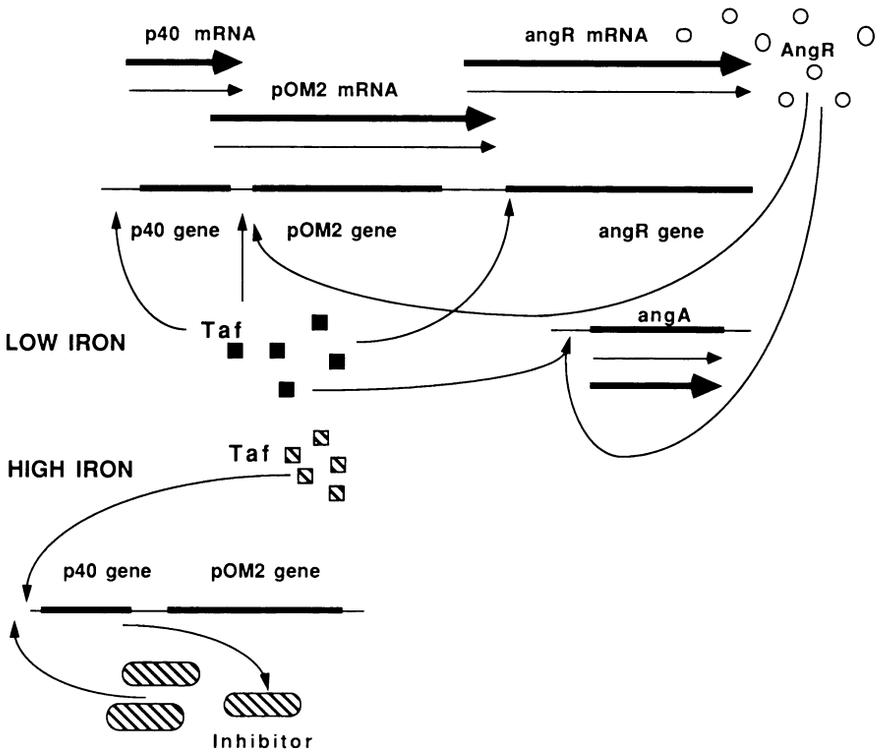

FIG. 3. Model of the regulation of the pJM1-mediated iron uptake system. Transcriptional activation of various genes is symbolized by the transition from a thin to a thicker arrow. The different symbols used for the Taf product acting under either iron-poor or iron-rich conditions indicate either that different Taf products are produced under these conditions or that Taf can be modified by iron to also act as an activator under iron-rich conditions.

pJM1-like plasmids but that strains originating in diseased fish from the Atlantic coast carried plasmids encoding an increased siderophore production phenotype, while those in fish from the Pacific Ocean behaved as the 775 strains (103). For example, one of the Atlantic Ocean strains, 531A, which carries the pJM1-like plasmid pJHC-1, produced three- to fourfold the amount of siderophore produced by strain 775. The gene associated with the increased siderophore production, designated *angR*, was mapped by using transposition mutagenesis and complementation experiments to within a 3.6-kb DNA region downstream of the transport genes (87a). It was remarkable that clones from pJM1 (from strain 775) and pJHC-1 (from strain 531A) carrying this region were identical as assessed by mapping with various restriction endonucleases and expressed a 110-kDa polypeptide in an *E. coli* maxicell system. However, only the clones from pJHC-1 enabled *V. anguillarum* cells containing a mutation in the *angR* gene (mutant 16) to produce siderophore at the level of strain 531A. Therefore, the *angR* region from strain 531A has a subtle difference, not detectable by restriction endonuclease analysis, at the nucleotide level from the similar region from strain 775. Full expression of the *angR* gene required a 2.9-kb DNA upstream DNA region in *cis*. Gene substitution experiments showed that this *cis* element, although essential, was not responsible for the difference in anguibactin production by strains 531A and 775. These experiments, together with the analysis of deleted derivatives, identified a downstream region of *angR* in the 531A clone that is essential for the increased siderophore phenotype; i.e., to achieve increased production of anguibactin, it is immaterial where the *cis* region comes from as long as the downstream region originates in the 531A clone. Therefore, the nucleotide sequence difference between the two clones is within the coding region of the AngR protein. Nonetheless, since the protein is present in clones derived from both 531A and 775 and since mutations or deletions in the *angR* gene lead to an anguibactin-deficient phenotype, it was obvious that it must play a role in anguibactin biosynthesis. Analysis of *lacZ* fusions demonstrated that *angR* is a positive regulatory gene for anguibactin biosynthesis rather than a biosynthetic gene and that it acts at the level of transcription (87a). Therefore, in addition to Taf, AngR regulates anguibactin production, albeit in a more selective way; that is, not all the siderophore genes were equally affected by AngR action, whereas all the anguibactin biosynthetic genes examined were stimulated by Taf at the level of transcription. Each one of these two factors stimulated transcription of the *lacZ* fusions two- to threefold; however, their combined action led to a larger increase (87a). This stimulation was more dramatic when anguibactin production, as the product of all the biosynthetic genes combined, was measured instead of β-galactosidase production. The results clearly showed that from an individual stimulation of about 3-fold, anguibactin production was increased about 24-fold when both the *angR* and *taf* transactivator genes were present (87a). Analysis of messenger ribonucleic acid (mRNA) specific for anguibactin biosynthetic genes (represented as angA in Fig. 3) confirmed that the presence of these two factors resulted in the cooperative enhancement of specific RNA synthesis at the transcription initiation step (87a).

This synergistic behavior closely resembles the cooperative promiscuity described for eucaryotic gene transcriptional activators such as the GAL4 yeast activator and mammalian transcriptional activators such as activating

transcriptional factor and upstream stimulating factor. Either of these activators used separately stimulated transcription perhaps 2- to 3-fold, whereas working together they stimulated transcription more than 50-fold (82).

Figure 3 shows a model of the pJM1-mediated iron uptake system of *V. anguillarum* that fits our present findings. Further work is required before we can dissect the precise mode of action of the positive and negative regulatory factors described above, although preliminary sequence analysis of the *angR* gene shows that it possesses features, such as a DNA-binding region, that are consistent with its role as a transcriptional regulatory factor (D. H. Farrell, P. Mikesell, L. A. Actis, and J. H. Crosa, Gene, in press).

IRON UPTAKE SYSTEMS IN ENTERIC BACTERIA

There are four iron uptake systems in *E. coli* that use siderophores such as enterobactin and aerobactin, produced by *E. coli* or the fungal siderophores ferrichrome and coprogen. In addition, iron transport in *E. coli* can occur in a process mediated by citrate (81). Each one of these systems needs a specific outer membrane receptor protein and various other membrane proteins, in general located in the cytoplasmic membrane. All of these systems are under the control of the Fur repressor gene, which will be discussed later in this review. In addition to the specific systems for the transport of ferric iron, there is a ferrous iron transport system that is expressed in *E. coli* cells if grown under anaerobic conditions. This system is specified by the *feo* gene and is also regulated by the Fur repressor protein (47).

The colicin I receptor protein encoded by the *cir* gene is another outer membrane protein synthesized by *E. coli* in response to iron limitation. No function in siderophore transport has been ascribed to the *cir* gene as yet (42, 70). However, it was recently reported that the presence of this protein substantially lowers the minimal inhibitory concentration of catechol-substituted cephalosporins, and there is an implication that it serves to transport ferri-monocatechols (30). In that work, evidence was presented that the transport is also dependent on the presence of a functional *tonB* gene (30). The *cir* gene has been shown to possess an operator spanning 43 to 47 bp, completely encompassing the two promoters P1 and P2 (42). It was exciting that this operator was identified as the binding site for Fur by using gel retardation and footprinting assays (42).

In this part of the review I will be concerned only with the *E. coli* siderophore systems in which the siderophore is produced by the bacterium that actually utilizes it to scavenge the iron, namely, the enterobactin and the aerobactin iron assimilation systems. Several publications have analyzed in detail the systems leading to the utilization of iron bound to fungal siderophores and to citrate (13, 26, 51, 54, 57, 68–71, 81, 113).

The *tonB* product is essential for all the ferri-siderophore uptake systems; mutations in *tonB* abolish not only ferrichrome uptake, but also all the other ferri-siderophore uptake systems (48). TonB, a 36-kDa protein also required for vitamin B_{12} uptake, is believed to be located in the cytoplasmic membrane (80). Genetic data demonstrated that the TonB protein interacts with the outer membrane receptors (13, 48). Furthermore, TonB-dependent outer membrane proteins have common amino acid sequences, suggesting that this region may be important for the interaction with TonB (54).

Mutants with mutations in *exbB*, like *tonB* mutants, are unable to take up iron (34, 46, 49), suggesting that the ExbB protein may play a similar role to the TonB protein. Ferric enterobactin and ferric citrate uptake also require *tonB* and *exbB*, together with the receptor protein genes *fepA* for ferri-enterobactin and *fecA* for ferric citrate and the *fepB* and *fecB* products, respectively (33).

Iron Release from Iron-Siderophore Complexes

Once the iron-siderophore complexes have been taken up by the outer membrane receptor protein and transported into the cell cytosol, iron can be released from the iron-siderophore complex by three mechanisms. In one of them, the siderophore brings the iron to the cell membrane but does not penetrate into the cell cytosol. This mechanism has been found in plants and yeasts (68). Alternatively, the ferri-siderophore is transported into the cytosol, where dissociation of the metal involves chemical breakdown of the ligand itself. For enterobactin, an esterase specific for the ferric complex may hydrolyze the ligand during iron release (33, 74). Finally, the iron-siderophore complex could also be transported intact into the cell, with iron being released via a reduction step. During this process, ferric iron is reduced to the ferrous state; since the siderophore has little affinity for Fe^{2+}, iron is released (33, 74). This mechanism appears to be common to all iron uptake systems. During the release of iron via reduction, the ligand may or may not undergo chemical modification. For example, enterobactin and ferrichrome are acetylated during iron release and are thus secreted into the external medium (51). In contrast, aerobactin is not destroyed after the release of iron: the free siderophore is once again excreted into the medium and reused in subsequent iron transport events (24).

Enterobactin-Mediated Iron Uptake

Iron limitation in *E. coli* and other enteric bacteria leads to the production of its native siderophore enterobactin (43, 71, 74, 79). An iron transport system is induced concomitantly with enterobactin; this consists of several proteins that intervene in the process of reception and internalization of iron (35, 70, 79). As stated above, in addition to the transport system specific for enterobactin, other transport systems specific for exogenous siderophores are induced (15, 16, 37, 68–71). This wealth of iron transport systems is controlled by the product of the *fur* gene (44–46).

In this section I will concentrate on the enterobactin system of *E. coli* and will relate its status in *Shigella* spp. Enterobactin (enterochelin) was first purified and characterized from *Salmonella typhimurium* and *E. coli* supernatants (74, 79). This siderophore belongs to the catecholate group and is synthesized in *E. coli* by a two-stage process. First, 2,3-dihydroxybenzoic acid is produced from the aromatic amino acid precursor chorismic acid, and then there is a subsequent conversion of 2,3-dihydroxybenzoic acid and L-serine into active enterobactin. The initial stage requires the products of three genes: *entC*, encoding isochorismate synthetase; *entB*, encoding 2,3-dihydro-2,3-dihydroxybenzoate synthetase; and *entA*, encoding 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase. The second stage entails the synthesis of one molecule of enterobactin from three molecules each of 2,3-dihydroxybenzoic acid and L-serine. A multienzyme complex composed of the products of the genes *entD*, *entE*, *entF*, and *entG* has been suggested as a catalyst for this step. The enterobactin gene cluster encompasses approximately 22 kb at 13 min on the *E. coli* chro-

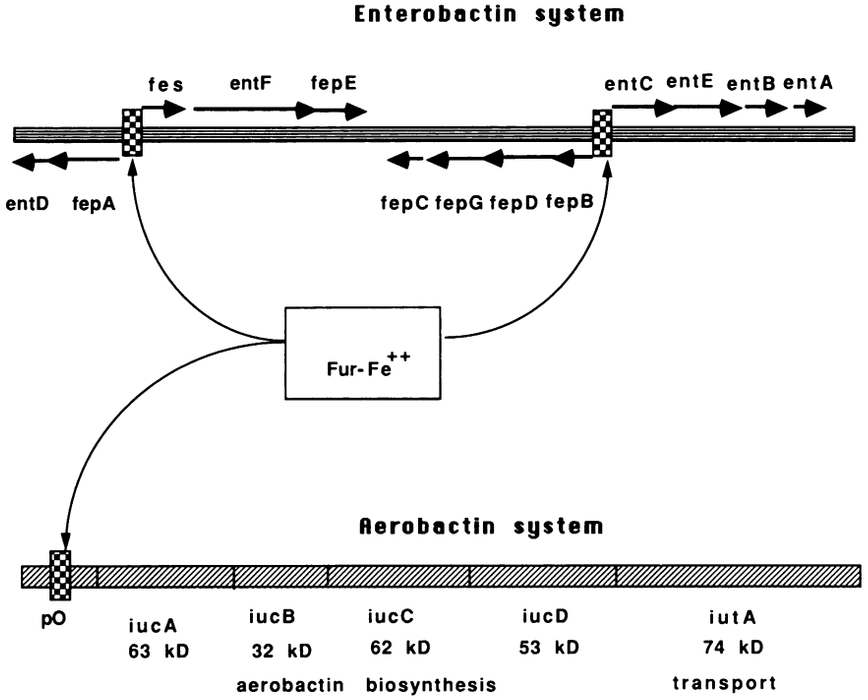

FIG. 4. Organization of the enterobactin and aerobactin systems and sites of interaction of the Fur-Fe^{2+} complexes. Symbol: ▩, iron boxes. The sites of Fur action on the enterobactin system are based on sequence analysis only.

mosome (33, 35, 67–71, 75; M. F. Elkins and C. F. Earhart, FEMS Microbiol. Lett., in press; G. S. Pettis, T. J. Brickman, and M. A. McIntosh, J. Biol. Chem., in press).

It was recently shown that when a stable insertion mutation was used between *fepB* and *entE*, production of 2,3-dihydroxybenzoic acid, the catechol precursor of enterobactin, was eliminated (67, 75; Pettis et al., in press). These investigators also demonstrated that this mutation disrupts the structural gene for a previously identified 44-kDa protein. Analysis of the nucleotide sequence of this gene identified similarities with other genes, such as *trpE* and *pabB*, encoding chorismate-utilizing proteins (75; Elkins and Earhart, in press). It is obvious from these results that the locus of the gene for isochorismate synthetase is indeed *entC* and that these enzymes may constitute a family of related proteins, possibly with a common evolutionary origin. Recent extensive sequence analysis through this region led to the identification of *entA* and *entB* (56, 67) and to the purification of the *entA* product, 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase, as an octamer of native molecular weight 210,000 (56). DNA sequencing analysis of 2,318 bp of DNA led to the identification of an ORF encoding an as yet uncharacterized protein, P15. By using an *entC*::*kan* mutant strain combined with DNA sequence and gene fusion data, it was established that the right-hand gene cluster is organized as an operon with five genes, *entC*, *entE*, *entB*, *entA*, and *P15* (67).

Primer extension analysis led to the identification of the *entC* transcription initiation site to about 55 bp upstream of the translation initiation codon for the EntC protein. A sequence related to the consensus Fur-binding site was found within this region. Although the actual DNA-binding experiments with Fur have not been carried out as yet, it is tempting to speculate that this sequence may actually contribute to the iron regulation of the expression of the *entCEBA*(*P15*) operon (Fig. 4). The *fepB* transcript is initiated upstream of the *entC* gene but on the opposite strand. The −10 and −35 promoter sequences for the two divergent transcripts are located in the 103 bp that separates the initiation sites for these two mRNAs. It is of interest that a similar situation occurs on the other end of the enterobactin cluster between the *fepA* and *fes* genes (Pettis et al., in press). In the latter case, the intercistronic region also serves as the starting point for divergent mRNAs, although with more overlapping of the −10 and −35 promoter sequences and with only 18 bp separating the primary initiation sites. The features of this region also include possible Fur-binding sequences. Both the *fepB* and *fepA* mRNAs possess sequences with the potential for extensive secondary structure formation, which could play a role in modulating the expression of these genes posttranscriptionally. The diagram in Fig. 4 shows an schematic map of the enterobactin cluster genes, the transcriptional units, and the potential Fur-binding sites.

Transport of ferric enterobactin into the cell cytosol requires the products of at least five additional genes (33, 75). One of these genes, *fepA*, has been studied in detail and corresponds to the gene for the outer membrane protein receptor for complexes of iron and enterobactin. Another of this set of iron transport genetic determinants is *fes*, which is required for the intracellular release of iron from enterobactin. The other genes are *fepB*, *fepC*, *fepD*, *fepE*, and *fepG*. The order of the enterobactin biosynthetic and iron transport genes in the *E. coli* chromosome is *entD-fepA-fes-fepE-fepC-fepG-fepD-fepB-entC-entE-entB-entA*.

The enterobactin genes are found in other members of the family *Enterobacteriaceae* such as *Salmonella*, *Klebsiella*, and *Shigella* spp. However, only 10% of the *Shigella* strains possess an active enterobactin iron transport system (76, 77, 89). Restriction endonuclease cleavage maps of cloned *Shi-*

gella flexneri enterobactin genes showed several restriction site differences between the *E. coli* and the *S. flexneri* systems (89). The existence of Ent$^-$ strains of *S. flexneri* was also discussed in that work. There is an IS*1* element near the 3′ end of the *entF* gene in the Ent$^-$ strains. However, it is not clear whether this IS*1* element has any effect on the expression of the enterobactin system. It is of interest that both the Ent$^+$ and Ent$^-$ strains possess the aerobactin genes originally found in the pColV-K30 plasmid and that these genes are fully derepressed in an Ent$^-$ *Shigella fur* mutant, whereas the *entB* gene remained repressed under both high- and low-iron conditions and the *entF* gene showed only a partial derepression (89). This aberrant expression of enterobactin genes in the Ent$^-$ *Shigella* strains could be due to the absence of a positive-acting factor or to the presence of a novel repressor which may act specifically at enterobactin regulatory regions, independently of the iron concentration of the cell (89). An alternative explanation given by Schmitt and Payne (89), is that the Ent$^-$ *Shigella* strains possess defects on the enterobactin promoters which may cause the reduced expression of these genes.

Aerobactin Iron Assimilation System

The aerobactin iron assimilation system was originally found in the pColV-K30 plasmid (39, 96, 112, 115). Members of our laboratory and that of S. Payne (59, 105) demonstrated that it can also be found in the chromosome of pathogenic bacteria such as invasive *E. coli* K-1, *S. flexneri*, and enteroinvasive *E. coli* (105–107). We determined that in clinical strains in which the aerobactin system is contained on a plasmid, the aerobactin genes were always adjacent to a replication region, REPI, and close to the partition genes; this led to our coining the designation "replication-virulence unit" (78, 110). This natural chimera must have played an important role in the preservation of the aerobactin system as a plasmid, since deletion or integration events protecting replication and maintenance regions would very probably result in the inclusion of the aerobactin genes in the newly generated replicons.

We have also described the presence, in aerobactin-producing strains of *Enterobacter aerogenes*, of a plasmid-mediated aerobactin system sharing partial homology with the pColV-K30 prototypic system (111) and have shown that strains of *Enterobacter cloacae* can harbor an aerobactin system that is totally unrelated to that of pColV-K30 (29). Therefore, the universal appearance of aerobactin as a siderophore in all these pathogenic bacteria, independently of the genomic status or of any divergence of the genes from the pColV-K30 standard, suggests that selective evolutionary pressures have worked on preserving the function rather than the genes themselves, underscoring the importance of aerobactin as a virulence factor.

The pColV-K30-type aerobactin-mediated iron assimilation system consists of five genes that are regulated as one operon by the iron status of the cell via the product of the chromosomal *fur* gene, a universal regulator of all iron transport systems in *E. coli* and possibly other bacteria (Fig. 4). The pColV-K30 genes involved in the biosynthesis of aerobactin, a hydroxamate siderophore, are *iucA*, *iucB*, *iucC*, and *iucD* (9). These genes encode polypeptides of 63 kDa (synthetase), 33 kDa (acetylase), 62 kDa (synthetase), and 53 kDa (oxygenase), respectively. In addition, the operon includes the gene *iutA*, which encodes a 74-kDa polypeptide that acts as the receptor for ferric aerobactin (9, 108, 115). The Fur protein acts as a repressor, using Fe^{2+} as a cofactor by binding to the operator of the aerobactin iron uptake system. Bagg and Neilands used purified Fur protein and a plasmid containing a *lacZ* fusion to the aerobactin operon in an in vitro-coupled transcription-translation system to demonstrate that the Fur protein requires Fe(II) or other divalent metals to regulate negatively the expression of the aerobactin operon (8, 9) (see next section).

The aerobactin genes from *Enterobacter aerogenes* and *Enterobacter cloacae* have recently been cloned in my laboratory and are at present being characterized to assess the nature of the divergent enzymes that still lead to the biosynthesis of an aerobactin molecule which, by physical analysis, is indistinguishable from that encoded by pColV-K30. Experiments to assess the regulatory circuits and possible existence of Fur-like proteins in these bacteria are presently under way. In the case of the *E. coli* K-1 chromosome aerobactin genes, we have nucleotide sequence and *lacZ* fusion evidence to suggest the presence of Fur proteins that have different affinities depending on the origin of the aerobactin system (106). As is the case for the enterobactin system, uptake of iron bound to aerobactin and other hydroxamate siderophores requires, in addition to the specific outer membrane protein receptor, a series of cytoplasmic membrane proteins. Thus, in the hydroxamate siderophore pathway, the specificity resides in the outer membrane receptor, whereas the cytoplasmic membrane components *fhuCDB*, *tonB*, and *exbB* are common to all three classes of hydroxamates (37). It is of interest that segments of the FhuC protein show very high homology to adenosine triphosphate-binding proteins (18). Therefore, it is possible that this protein functions in the uptake of ferric iron mediated by aerobactin and other hydroxamates in an analogous fashion to adenosine triphosphate-binding proteins of transport systems that require periplasmic proteins (18, 73).

Regulation of the Iron Uptake Systems in *E. coli*

Regulation of iron uptake systems in bacteria and fungi by the concentration of iron was first described by Garibaldi and Neilands in 1956 (38). It was proposed that the iron regulation was mediated through an iron-binding repressor protein which, under iron-rich conditions, inhibited the expression of genes required for siderophore biosynthesis and for their receptor proteins (68–71). Subsequently, a mutant was isolated that constitutively overexpressed iron-regulated outer membrane proteins. The mutation, termed *fur* (ferric uptake regulation), resulted in the constitutive production, transport, and degradation of enterobactin, as well as the constitutive uptake of ferrichrome (36, 44).

The *fur* gene has been sequenced and shown to encode a 17-kDa polypeptide rich in histidine (44–46, 88). The purified Fur protein and a plasmid containing a *lacZ* fusion to the aerobactin operon were used in conjunction with an in vitro-coupled transcription-translation system to demonstrate that the Fur protein requires Fe^{2+} or certain other divalent metals as cofactors to negatively regulate the expression of the aerobactin operon (8, 9).

In deoxyribonuclease I protection experiments with excess of divalent metal, in this case Mn^{2+}, increasing the level of Fur protein led to protection of sequences including the −35 region and a secondary site located downstream of the −10 region. These sites have in common the sequence ATAATnnnnATnATT (8, 9, 32). A similar sequence occurs upstream of the *fur* gene itself and in the promoter regions of the iron-regulated genes *fhuA* and *fepA*, which encode the receptors for complexes of ferric ferrichrome and ferric

enterobactin, respectively. By comparing all these sequences, a consensus for a palindromic box was derived as 5′-GATAATGATAATCATTATC and suggested as the putative recognition sequence for the ferrous Fur complex (32).

Fur was shown to bind to and block the aerobactin promoter in a metal-dependent fashion (9). The Hill plot generated from the in vitro binding experiments suggested that the Fur protein acts as a dimer in its interaction with iron on the aerobactin promoter (8). Deoxyribonuclease I footprinting analysis showed that the aerobactin promoter region has two contiguous binding sites of different lengths and affinities for Fur (32). The primary site for Fur binding spans 31 bp and contains two overlapping dyad symmetries which contain the sequence 5′-TCATT-3′ (32).

The recognition site for Fur protein, designated "iron box," has been found in several iron-regulated genes such as *fhuA*, *fepA*, *cir*, those encoding certain bacterial toxins, and those in the *fur* promoter region. Footprint analysis of the *fur* promoter region indicated that the Fur protein binds to its own promoter and autoregulates its expression (32).

Examination of the DNA sequences located upstream of the *fur* gene revealed a possible binding site for the catabolite gene activator protein (CAP). Analysis of β-galactosidase levels in *E. coli* cells harboring the *fur-lacZ* fusion were measured in *crp* (lack of catabolite gene activator protein) and *cya* (lack of cyclic adenosine monophosphate synthesis) genetic backgrounds. The results demonstrated that *fur* expression is regulated through the cyclic adenosine monophosphate-catabolite gene activator protein system (31). This type of regulation suggests a correlation between the modulation of iron absorption and the metabolic status of the cells.

I will end this section by reporting what I believe is another example of the universality of regulation by *fur*. Superoxide dismutases are metalloproteins that catalyze the disproportionation of superoxide anions. There are three forms of superoxide dismutases: an iron-requiring protein, which is found in bacteria and plants; a manganese-harboring protein, which is found in bacteria; and a copper-zinc protein, which is found in eucaryotic cells (19, 99). Given the similarity in physical and catalytic properties, it was hypothesized that superoxide dismutases may have a common biological function, i.e., protection against oxygen toxicity. Recently, investigators in the laboratory of J. A. Fee found that in *E. coli* the *fur* locus regulates the two *sod* genes, *sodA* and *sodB*. Niedhoffer et al. (72) identified a sequence in the promoter region of the *sodA* gene which bears a strong resemblance to the iron box sequence of other genes controlled by *fur*; by contrast, such a sequence was not observed in the promoter region of *sodB* (19). Unexpectedly, it was found that iron superoxide dismutase synthesis, a measure of *sodB* activity, was dramatically decreased in Fur^- cells whereas manganese superoxide dismutase synthesis, an indication of *sodA* expression, was little affected (72). More recently, it has been shown that transcription of *sodA* is repressed by metallated (holo) Fur under certain growth conditions, whereas transcription of *sodB* is positively regulated by demetallated (apo) Fur (72). Moreover, preliminary evidence indicates that $SodB^-$ cells grown under low-iron conditions are significantly derepressed in enterobactin biosynthesis, suggesting that SodB and Fur may act together, in a complex fashion, to control the biosynthesis of enterobactin (72).

SIDEROPHORE SYSTEMS IN *PSEUDOMONAS* AND *AEROMONAS* SPP.

Pseudomonas spp. are of considerable importance both in agriculture and as human pathogens. Two important siderophore-mediated iron uptake systems have been found in these bacteria: one involving the fluorescent siderophore pseudobactin (also known as pyoverdin) and the other involving the siderophore pyochelin. These siderophores may be important virulence factors for this organism. For instance, it has been reported that pyochelin stimulates bacterial growth in murine infections (22). Furthermore, both siderophores interact effectively with transferrin, the major component of nutritional immunity (5). It was demonstrated that both siderophores promote the removal of iron from transferrin (94) and the growth of mutants defective in siderophore production. Mutants defective in ferripyochelin transport were markedly less virulent than wild-type strains (64, 90, 92). Furthermore, these siderophores could affect the availability of iron to the cell and thus influence the production, or lack of production, of toxin A, alkaline protease, and other virulence factors that are regulated by the concentration of iron in the cell cytosol (11, 12, 91). Pyochelin is a phenolate siderophore: 2-(2-(*o*-hydroxyphenyl)-2-thiazolin-4-yl)-3-methyl-4-thiazolidine carboxylic acid (Fig. 2), and the stoichiometry of iron binding appears to be two pyochelin molecules to one of Fe^{3+}. This compound has a low molecular weight (of 324) and a very low iron-binding coefficient (5×10^5) (23). Despite this low iron-binding coefficient, pyochelin is very active in iron transport and growth stimulation in media containing transferrin and has been implicated in the pathogenicity of *Pseudomonas aeruginosa* (21). Pyochelin was recently synthesized in the laboratory, and the synthetic product was indistinguishable from natural pyochelin in terms of both chemical and biological activities (6).

Under iron-limiting conditions, fluorescent pseudomonads also produce the yellow-green fluorescent siderophores known a pyoverdins. Pseudobactin 358 is one of these siderophores; it is produced by the rhizosphere-colonizing *P. putida* WCS358 (61). Pseudobactin 358 has a relatively high affinity for Fe^{3+}, the iron-binding coefficient being 2×10^{25} at pH 7.0. *P. putida* WCS358 is important to agriculture because it can reduce crop yield losses caused by bacteria and fungi in the root environment. The protective activity is related to the production and excretion of siderophores which efficiently chelate the iron in the root environment. This iron deficiency leads to an impaired growth of the deleterious microorganisms. Siderophore-defective mutants obtained by transposition mutagenesis were unable to induce plant growth stimulation.

The pyoverdins are chromopeptides that have a peptide chain of 6 to 10 amino acids bound to a chromophore related to 2,3-diamino-6,7-dihydroxyquinoline (100). The structure of one of the pyoverdins, pseudobactin, was solved by X-ray diffraction analysis several years ago, and now the complete structures of the pyoverdin of *P. aeruginosa* and other pyoverdins have been reported (114). Transposition mutants defective in the biosynthesis of pseudobactin have been described (61). Complementation of these mutants with cosmid clones generated from a genomic library of strain WCS358 led to the identification of at least five gene clusters associated with the biosynthesis of pseudobactin. Recently, the transcriptional organization of one of these gene clusters involved in the iron-regulated biosynthesis and transport of pseudobactin 358 was analyzed (60). This region is the major

gene cluster, extends for approximately 33.5 kb, and encodes at least five transcripts of various sizes. Analysis in *E. coli* minicells and sequencing experiments have led to the identification of large ORFs and their encoded polypeptides in these transcripts and to the characterization of several promoter regions (60). At least two of the genes showed an iron-dependent expression which appeared to be regulated at the transcriptional level. Other investigators have previously shown that several gene clusters are involved both in the production of a fluorescent compound in *Pseudomonas* spp. and in the biosynthesis of pseudobactin (58, 65). One of the gene clusters in *Pseudomonas* strain B10 harbored the gene encoding the outer membrane receptor protein which was present in a separate operon and was flanked on both sides by biosynthetic genes. It is of interest that in the different fluorescent *Pseudomonas* strains, the biosynthetic genes for pyoverdin are dispersed around the genome, although, as described above, clustering of genes in large regions can also be found.

A. hydrophila and other members of the *Aeromonas* genus are fish and human pathogens, causing fatal hemorrhagic septicemias in the former and blood, wound, and soft-tissue infections and gastroenteritis in the latter. A new siderophore, amonabactin, was recently reported to be excreted by *A. hydrophila* 495A2 (10). This siderophore was produced in two forms, which were composed of 2,3-dihydrobenzoate, lysine, and glycine; one form (amonabactin T) also contained tryptophan, whereas the other (amonabactin P) contained phenylalanine instead of tryptophan. These two siderophores behaved differently in thin-layer chromatography on polyamide and could be separately purified by a combination of chromatographic and precipitation methods. Amonabactins T and P were produced simultaneously throughout the growth cycle of *A. hydrophila*, and both were biologically active, stimulating growth of siderophore-deficient mutants in iron-deficient medium. It was of interest that addition of tryptophan to the medium greatly lowered amonabactin T production and completely abolished amonabactin P synthesis, suggesting that the two forms of the siderophore were synthesized by means of a single biosynthetic system. Amonabactin may be able to extract iron from transferrin, although it is not yet known whether amonabactin might be an important virulence factor in *Aeromonas* species. The original reports concluded that certain *A. hydrophila* strains produced enterobactin; however, many isolates are now known that produce amonabactin: of 25 *A. hydrophila* isolates (10), more than 70% produce amonabactin, whereas the remainder produce phenolates and some may be enterobactin producers.

CONCLUDING REMARKS

I hope that it is now apparent that the genetic makeup needed for the utilization of environmental iron can range from a very simple operonlike organization, such as in the aerobactin system, to the very complex regulatory circuitry found in the *V. anguillarum* plasmid-mediated iron uptake system, in which positive and negative regulatory factors control the biosynthesis of siderophore and membrane transport proteins involved in iron translocation into the cell cytosol. Recent experiments with *V. anguillarum* suggest that iron-mediated control of the production of siderophore is tighter than that of the membrane transport complex (P. C. Salinas and J. H. Crosa, manuscript in preparation). This is somewhat as expected, since under relatively comfortable high-iron concentrations the cell should be ready to utilize any wandering siderophore, whereas it should not be wasting energy in synthesizing all of the components of the siderophore biosynthetic pathway.

It should also be clear that although siderophores and iron transport appear to contribute to the virulence repertoires of all the microorganisms discussed above, this should not be taken as the rule. For instance, production of the *V. cholerae* siderophore vibriobactin is apparently nonessential for infections of the intestinal mucosa by this bacterium, and I stated at the beginning of this review that *N. gonorrhoeae* and *N. meningitidis* use iron directly from transferrin or lactoferrin without the agency of a siderophore. This is also the case for *Haemophilus influenzae*, which utilizes transferrin (but not lactoferrin) (52a). Of course, it is well known that other microorganisms obtain iron from its association with heme and thus utilize hemolysins to lyse erythrocytes and liberate this metal. Either or both of the hemolysin and aerobactin iron uptake determinants are always found in invasive *E. coli* K-1 strains, which cause human neonatal infections (107). Increased levels of hemolytic activity were found in supernatants of iron-starved cultures of El Tor and non-O1 strains of *V. cholerae*; synthesis of hemolysin was found to be iron regulated in these isolates (95). It is of interest that spontaneous hemolysin-deficient mutants, which occur at high frequency, failed to synthesize vibriobactin, the *V. cholerae* siderophore, whereas constitutive mutants for the production of hemolysin were also constitutive for the biosynthesis of this siderophore. When a plasmid containing the cloned *fur* gene was introduced into this constitutive strain, normal iron regulation of both vibriobactin and hemolysin was regained (95).

Another paramount mechanism of virulence in which iron plays a role is that of regulating the biosynthesis of important toxins and other extracellular virulence factors in certain bacteria, such as toxin A and proteases in *P. aeruginosa* and diphtheria toxin in *Corynebacterium diphtheriae*. The synthesis of these toxins is inhibited by iron, and transcription of their genetic determinants is decreased under iron-rich conditions (11, 12, 26, 66). The iron uptake systems present in *Pseudomonas* spp. were discussed earlier in this review. Therefore, I will give some insights into the mechanism of iron uptake in *C. diphtheriae* and its relationship to toxin production. The iron uptake system of this bacterium is, as expected, induced under conditions of iron limitation, and mutants affected in iron uptake have been isolated. These mutants are, in general, also affected in terms of iron regulation of toxin production. One of the mutants, defective in the production of the corynebacterial siderophore, is best known as strain PW8, which is a very highly toxinogenic strain of *C. diphtheriae* (86, 87). Furthermore, recent evidence suggested that the cloned diphtheria toxin promoter is iron regulated in *E. coli*. When a plasmid containing a *tox-galK* fusion was introduced into a *fur* mutant of *E. coli*, expression of the galactokinase was independent of the iron status of the cells, but repressibility by iron was restored upon introduction of a second plasmid carrying the *fur* gene (98). It was remarkable that a subregion of the diphtheria toxin promoter indeed shows homology with the consensus sequence found in other iron-regulated promoters and on the *fur* gene itself (98).

The prediction of Murphy and Bacha (66) that the *tox* operon of *C. diphtheriae* is negatively regulated by a chromosomally mediated repressor protein that uses iron as a corepressor is now supported by strong molecular evidence. A Fur-like product that regulates diphtheria toxin production

in cooperation with iron must therefore be present in *C. diphteriae*.

I would like to finish this review by calling attention to a remarkable structural, and possibly physiological, similarity in the iron-regulated transcripts of the *V. anguillarum* iron transport proteins and those of transferrin and ferritin in eucaryotes.

Iron enters the cells of the body from transferrin, a glycoprotein normally about one-third saturated with Fe(III). Transport of iron from iron-transferrin complexes depends on a transferrin surface receptor (TfR), which consists of two disulfide-linked proteins. Increased iron concentration raises the synthesis of ferritin and represses the biosynthesis of TfR. Ferritin is regulated at the translational level (52). Under iron stress conditions, the majority of ferritin mRNA is associated with cytoplasmic proteins (mRNP). An increase in the intracellular iron concentration produces a mobilization of ferritin mRNA from mRNP to polyribosomes and a concomitant translational activation. A *cis*-acting element located at the 5′ end of the ferritin mRNA is responsible for iron regulation. The regulatory sequence is called the iron-responsive element. In contrast, TfR synthesis is controlled at the transcriptional and posttranscriptional levels (20, 84). A locus located at the 5′-flanking region of the gene is responsible for the control at the level of transcription. A much larger effect by iron is mediated through a region located at the 3′ end of the TfR mRNA. This region contains elements similar to those found on the ferritin IRE (20, 52). It has been proposed that IRE functions by forming a stem-loop structure in the RNA, which is recognized by a specific factor (20, 52). According to this model, a *trans*-acting factor binds to the IRE at low iron concentrations. If the IRE were located at the 5′ end (as occurs in the ferritin mRNA), there would be a block of translation. However, binding of the factor at the IRE located at the 3′ end of the Tfr RNA resulted in increased mRNA stability (52).

Our recent sequencing results with the *V. anguillarum* pOM2 protein gene (3) showed the presence of a stem-loop structure right at the 3′ end of this gene. Mutations adjacent to this region resulted in complete suppression of the biosynthesis of pOM2, the iron-regulated outer membrane protein, whereas downstream deletions led to pOM2 biosynthesis that was independent of the iron status of the cell. This structure may therefore be involved in the iron regulation of this bacterial system, as is the case for the IRE of the transferrin transcripts.

These remarkable similarities between the procaryote and eucaryote iron transport systems underscore the importance of these findings with respect to the host-bacteria interactions leading to disease. An increased knowledge of the molecular mechanisms of microbial pathogenicity mediated by iron and host resistance will undoubtedly result from the studies of these systems. However, it is likely that bacteria will still have wonderful surprises awaiting us.

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