Research Communication

Interplay Between Siderophores and Colibactin Genotoxin in *Escherichia coli*

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Abstract

Highly pathogenic *Escherichia coli* strains that belong to the phylogenetic group B2 have developed a greater ability to acquire iron (heme receptor and numerous siderophores), to produce the genotoxin colibactin and to synthesize antimicrobial siderophore-microcins. There is an increased prevalence of these *E. coli* strains over the last 30 years in the intestinal microbiota in industrialized countries. Integrating the regulation of fitness/virulence factors, such as siderophores, colibactin and siderophore-microcins into networks that respond to specific environmental signals, such as the local iron concentration, could result in an accurate production of specific fitness/virulence factors, so that the *E. coli* can adapt to the competitive environment that is the gut and/or the blood. Iron deficiency is common in infancy, even in industrialized countries. Usual strategies for anemia correction are iron supplementation and iron fortification of foods. The long-term consequences and risks associated with high iron supply in the light of this iron-dependent network described in this review could explain at least in part the increased prevalence of *E. coli* B2 in the gut of people in industrialized countries. © 2017 IUBMB Life, 69(6):435–441, 2017

Keywords: *pathogenesis; commensalism; iron;* E. coli

Battle for Iron Between Host and Microbes

Eukaryotic cells and most prokaryotic organisms require iron to maintain essential biological functions (e.g., oxygen transport, energy metabolism, DNA synthesis). Intense competition for iron between host and bacterial pathogen occurs during the course of an infection. Microbes have evolved various mechanisms to acquire iron during infections of higher organisms (1,2). In turn, the host immune system has the capacity to manipulate iron levels to limit the multiplication of pathogens (3).

In the human body, most of the iron is intracellular or sequestered by proteins, which makes it unavailable to invading microbes, and constitutes the first line of defense against bacterial invaders. Within hours of infection in humans, concentrations of iron in extracellular fluid and plasma dramatically decrease (4). The development of this hypoferremia results from the rise in hepcidin, which is the peptide hormone that controls extracellular iron concentrations by regulating dietary iron absorption and the release of iron from stores in macrophages and hepatocytes. This process, that is, “nutritional immunity”, is a critical host defense strategy against bacterial pathogens (5). This is consistent with the concept that infections with a variety of microbes are increased in frequency and severity in humans with iron overload (3,6). Moreover, hepcidin was reported to display microbicidal activity against many classes of microbes *in vitro* (6). Intense competition between bacteria and host for iron is also exemplified by the fact that mammalian neutrophils produce lipocalin-2, also called siderocalin (7), a molecule that binds the siderophore (see chapter 2) enterobactin and prevents enterobactin-mediated iron uptake by bacteria (8). Bacterial pathogens overcome this barrier by producing salmochelins, glycosylated derivatives of enterobactin, which is not recognized by lipocalin-2 (9). Uropathogenic *E. coli* (UPEC) represses hepcidin, the master regulator of host iron homeostasis, to evade

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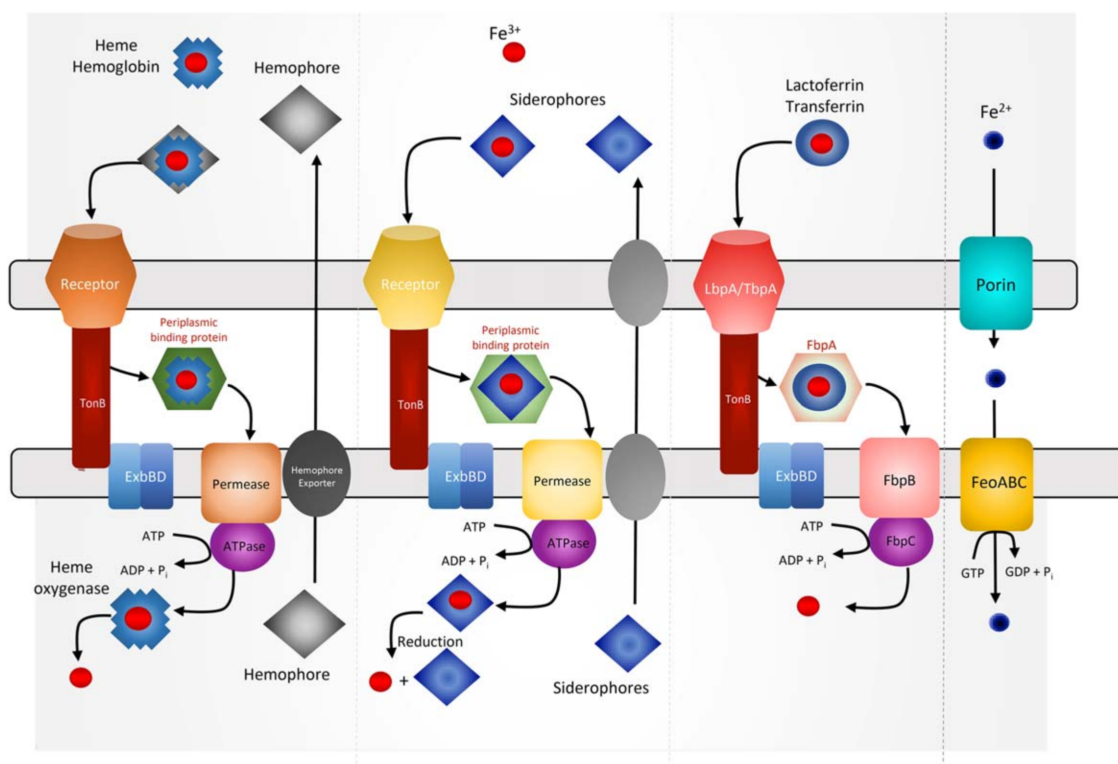

FIG 1 *A schematic view of iron uptake systems evolved by Gram-negative bacteria. Many bacteria secrete siderophores to bind Fe^{3+} and take it up by specific receptors that are energized by the Ton complex. Some pathogenic bacteria are able to use lactoferrin- or transferrin-bound iron either with the help of their siderophores or with specialized receptors in their outer membranes. Other bacteria have additional systems to use the heme-bound iron of their host either by secreting a special heme-binding protein, a hemophore, or through specific receptors for heme, hemoglobin, or hemopexin, which are able to extract the heme and transport it across the outer membrane. All these different substrates—ferric iron from transferrin, heme and Fe^{3+} siderophores—are transported by specialized ABC transporters across the cytoplasmic membrane. In the cytoplasm, Fe^{3+}-siderophore reductases or heme oxygenases help to mobilize the iron for cell metabolism. The FeoABC system can transport the more soluble Fe^{2+} through a not well-characterized mechanism.*

renal host defenses during urinary tract infection, likely by acting on the BMP6/SMAD pathway (10).

Siderophores, General Considerations

Because iron is an essential nutrient for their survival, bacteria have developed a sophisticated array of mechanisms that provide them with iron (Fig. 1). Bacteria produce and secrete siderophores in their environment and express at the level of the outer membrane specific transporters. These transporters are responsible of the uptake of ferri-siderophore complexes (11) (Fig. 2). Siderophores are crucial for bacterial survival and play a significant role in virulence of *Escherichia coli* (12), *Klebsiella pneumoniae* (13) or *Pseudomonas aeruginosa* (14).

Pathogenic bacteria frequently synthesize different siderophores with different chemical structures. They differ in their affinity for Fe^{3+} and are differentially expressed depending on the environmental context. They are not functionally redundant. Mobley's group demonstrated nonequivalent roles for distinct iron acquisition systems during urinary tract colonization by UPEC and provided evidence for a functional hierarchy of these systems (16). The inability to produce aerobactin only by *K. pneumoniae* results in an attenuated virulence phenotype in a diversity of models of infection (13). *P. aeruginosa* first produces pyochelin and switches to pyoverdine production only when the concentration of iron becomes really low (14). In chronic infections, such as in cystic fibrosis lungs, the production of pyochelin could play a role in the sustained inflammatory response which is known to occur and cause damage to tissues. On the other hand, pyoverdine, the high affinity siderophore, is needed to cause acute infections (14).

Siderophores in *E. coli*, Repertoire and Regulation

E. coli is a normal resident of the lower gut of humans and animals. Although usually a commensal, *E. coli* can also be a pathogen, associated with diarrheal diseases and extraintestinal infections (17,18). The majority of *E. coli* strains can be assigned to one of seven main phylogenetic groups A, B1, B2, C, D, E and F (19). Strains of the distinct phylogenetic groups differ in their phenotypic and genotypic characteristics

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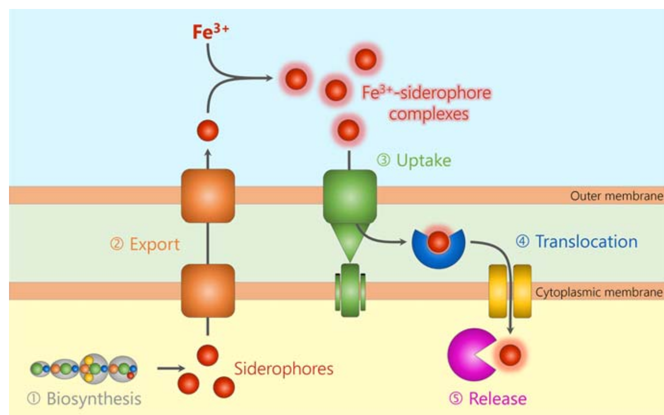

FIG 2 *Siderophore-dependent iron uptake. Bacteria rely on high-affinity surface receptor proteins (green) that bind iron-loaded siderophores. In* E. coli *and many other Gram-negative bacteria, ABC transporters associated with periplasmic binding proteins import ferri-siderophores across cytoplasmic membranes. In some siderophore pathways, this step can also be carried out by proton-motive force-dependent permeases (15). Periplasmic-binding proteins (blue) and ATP-driven transporters (yellow) that are in the cytoplasmic membrane are used to ensure further transport into the cell.*

(20–22). Extraintestinal pathogenic *E. coli* (ExPEC), which mainly belong to the B2 group, and which display enhanced ability to cause infection outside the intestinal tract, carry specific genetic determinants that are clustered on different pathogenicity islands (23). These virulence factors associated with extraintestinal infections are nonrandomly distributed, and strains of the *E. coli* phylogenetic group B2 harbor the greatest frequency and diversity of virulence traits (24,25).

E. coli synthesizes up to four types of siderophores: enterobactin, salmochelins (that are glycosylated forms of enterobactin), yersiniabactin and aerobactin (Fig. 3; (26)). If all *E. coli* strains produce at least one siderophore, that is, enterobactin, the analysis of the repertoire of siderophores displayed by *E. coli* reference strains revealed that B2 strains cluster the highest number of distinct siderophores (Fig. 4; (27)).

Each siderophore has specific affinity for iron: the range of siderophore association constant for Fe^{3+} lies between 10^{12} and 10^{52} (33). Siderophores may be differentially regulated to provide different advantages, potentially allowing ExPEC to adapt to different environmental conditions or to overcome host innate immunity (8,34–36).

Enterobactin, salmochelins and yersiniabactin are nonribosomal peptides (NRP) or PK (polyketide)-NRP hybrids (37). Their biosynthesis requires a 4'-phosphopantetheinyl transferase (PPTase). PPTases activate polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs) by catalyzing the transfer of a phosphopantetheinyl (P-pant) moiety from coenzyme A to conserved serine residues on PKSs and NRPSs (38). This results in the conversion of inactive apo-synthases to active holo-synthases. In *E. coli*, the EntD PPTase is involved in the synthesis of enterobactin and salmochelins (35). The other siderophore necessitating a PPTase is yersiniabactin. This siderophore is encoded by the high-pathogenicity island (HPI) that was acquired through horizontal transfers (39). The HPI core region was detected in more than 70% of ExPEC isolated from blood cultures, urine samples and cerebrospinal fluid (40). While yersiniabactin production in *Yersinia* requires the YbtD PPTase encoded outside the HPI (41), no gene homologous to *ybtD* has been identified in the genome of *E. coli* strains producing yersiniabactin. We showed that the PPTase committed to the synthesis of yersiniabactin in *E. coli* was EntD (27).

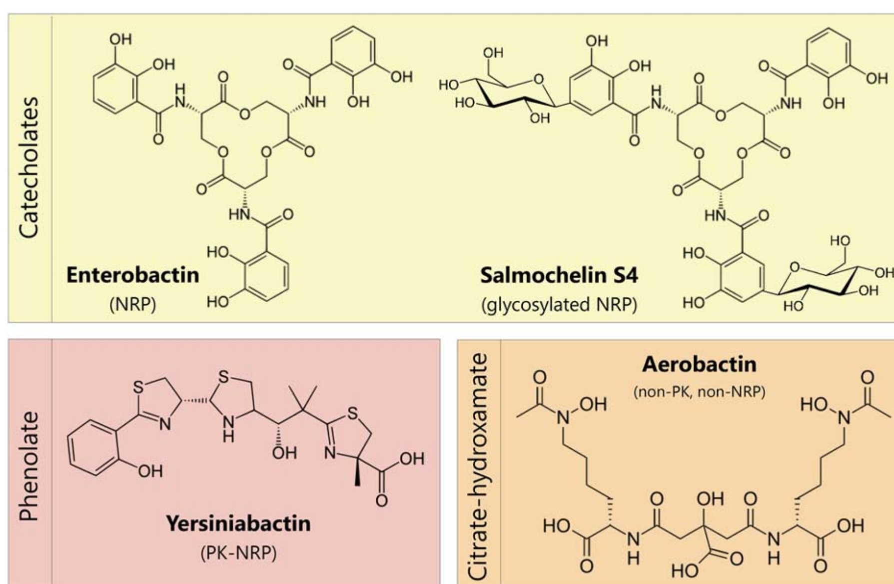

FIG 3 *Structure of the siderophores synthesized by* E. coli *(according to 26).*

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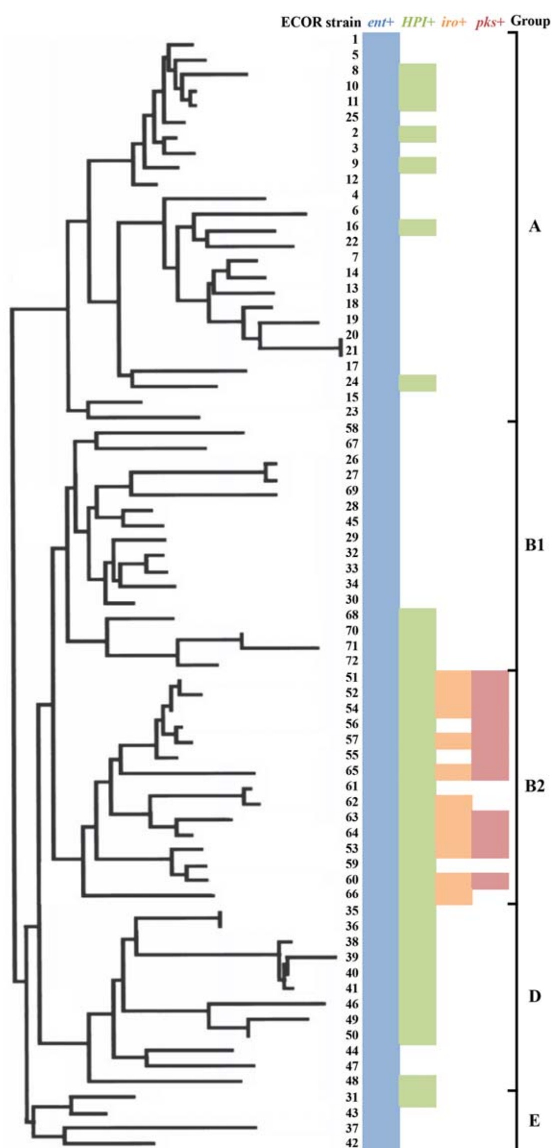

FIG 4 *Phylogenetic relationships among the* E. coli *reference strains (ECOR, (28,29)). The phylogeny was based on MLST of backbone genes (30,31). ent locus (enterobactin) positive strains are indicated in blue, HPI island (yersiniabatin) positive strains are indicated in green (32) and iro locus (salmochelins) positive strains are indicated in orange. The presence of the iro locus was determined only in B2 strains.*

The regulation of the expression of siderophores is highly complex, involves iron availability and relies on ferric uptake regulator (Fur) protein and the small regulatory noncoding RNA (sRNA) RyhB (42,43). However, in the same iron limited environment, distinct ExPEC strains were shown to display a distinct transcriptional profile of siderophores and a general coregulation of all iron uptake system is not observed (44). Moreover, inactivation of one, two, three or four siderophores results in highly differential global metabolomics responses (45). This implies the involvement of siderophores in various processes in addition to iron acquisition.

Interplay Between Siderophores and Colibactin Genotoxin Biosynthetic Pathways in *E. coli*

A number of *E. coli* strains from phylogenetic group B2 display also the *pks* island, which codes for the production of colibactin, a PK-NRP genotoxin (45) that belongs to the same family of chemical compound as enterobactin, salmochelins and yersiniabactin. The synthesis of colibactin also requires a PPTase encoded by the *clbA* gene located on the *pks* island. Colibactin induces DNA double-strand breaks, cell cycle arrest in G2-phase and megalocytosis in infected eukaryotic cells. *E. coli* strains harboring the *pks* island can induce DNA damage in enterocytes *in vivo* and trigger genomic instability in mammalian cells (46). In a rodent model of inflammation, colibactin potentiates the development of colon cancer (47,48). Colibactin is also required for the anti-inflammatory properties of the probiotic *E. coli* strain Nissle 1917 (49). Epidemiological studies revealed that the vast majority of the colibactin-positive *E. coli* strains were clinical ExPEC and that the *pks* island was significantly associated with a highly virulent subset of ExPEC isolates (50). Strikingly, an analysis of the prevalence of the colibactin island among *Enterobacteriaceae* revealed that the *pks* island was constantly associated with the yersiniabactin gene cluster (51).

A potential interplay between the biosynthetic pathways leading to the production of siderophores and colibactin, through a possible functional interchangeability between PPTases was investigated. ClbA was reported to contribute to the synthesis of siderophores both *in vitro* and *in vivo*. In a mouse model of sepsis, the presence of either functional EntD or ClbA is required to maintain full virulence of ExPEC (27). This evidenced the interconnection between pathways leading to the synthesis of siderophores and genotoxin, via the PPTase ClbA (Fig. 5; (27)). Therefore, the strict association of the *pks* island with HPI could have been selected in highly virulent *E. coli* isolates because ClbA can contribute to the synthesis of both the genotoxin and yersiniabactin.

Moreover, we recently highlighted the role of the molecular chaperone HtpG (or $Hsp90_{Ec}$), the bacterial homolog of eukaryotic heat shock protein 90, in the production of both colibactin and yersiniabactin (52). The ClpQ protease was shown to be involved in colibactin and yersiniabactin production in combination with $Hsp90_{Ec}$. These novel findings confirmed the interplay between the biosynthesis of two *E. coli* virulence factors, genotoxin colibactin and siderophore yersiniabactin. This also highlights that the synthesis of secondary metabolites is closely related to the primary metabolism, or, in other words, that genes of the core genome are required for the biosynthesis of pathogenic islands.

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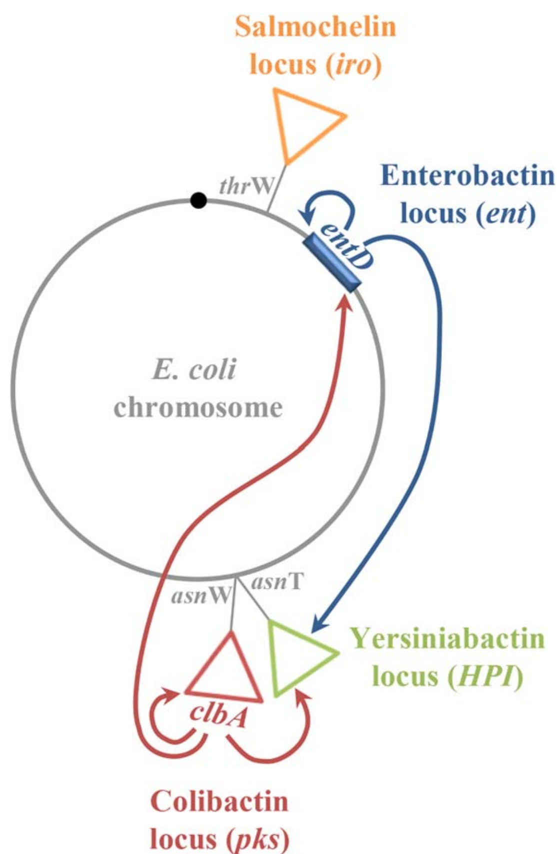

FIG 5 *The archetypal chromosome of phylogroup B2* E. coli *strains. The loci encoding enterobactin (*ent*), yersiniabactin (*HPI*), salmochelin (*iro*) and colibactin (*pks*) are represented. The arrows originating from PPTases EntD and ClbA and pointing toward other loci illustrate the capacity of the PPTase to contribute to the synthesis of metabolites from other loci.*

Iron Homeostasis Regulates the Genotoxicity of *E. coli* that Produces Colibactin

Given the existence of crosstalk between colibactin and siderophore biosynthesis (27,52), we speculated that iron could play a key role in this connection. The study we designed to address this question revealed that the expression of *clbA* and the production of colibactin were tightly controlled by iron bioavailability, through Fur and RyhB (53) (Fig. 6). Therefore, iron could constitute a key environmental factor contributing to the virulence of *E. coli* strains producing colibactin.

Iron deficiency is common in infancy, even in industrialized countries. Breast milk and cow milk naturally contain little iron. Improved growth, significant increase in hemoglobin levels and decreased anemia are associated with iron enriched diet in young children (54–56). However, the long-term consequences and risks associated with iron supplementation are not all known. Iron supplementation could indeed have side effects on health. Usual strategies for Fe-deficiency anemia correction are Fe supplementation and Fe fortification of foods. However, absorption of Fe is usually low (5–20%) and takes place mainly in the duodenum, while the main fraction of Fe reaches the colon, where it might affect the gut microbiota (57). For instance, anemic African children were reported to carry in their feces an unfavorable ratio of *Enterobacteriaceae* (potentially pathogenic) versus Bifidobacteria and Lactobacilli (beneficial bacteria). This ratio becomes even more unfavorable when children are fed iron-enriched food (58). Besides, oral iron supplementation could, in addition to inducing pathogenic overgrowth, also increase the virulence of enteric pathogens (59).

The fact that colibactin and siderophores are regulated by the two major regulators of iron homeostasis could make a link between iron concentration in the gut/the blood and *E. coli*-mediated carcinogenesis/systemic infections. Given the existence of a gradient of iron concentration from the lumen to the intestinal epithelial cell (60), we hypothesize a fine-tuning of the production of colibactin and siderophores takes place when the pathogenic *E. coli* is located in an appropriate site in the gut. A variation of the local iron concentration together with the balance between Fur and RyhB could result in an accurate production of colibactin and siderophores, so that the bacteria can adapt to the competitive/harsh environment that constitutes the gut and the blood.

Siderophore-Microcines in *E. coli*

Microcins are ribosomally synthesized peptides that are involved in microbial competition within the intestinal tract. The gene clusters may be chromosomally or plasmids encoded and comprise two genes: the microcin gene and the immunity gene. Microcins exert potent bactericidal activities that use brilliant mechanisms to cross outer and frequently inner membranes of Gram-negative bacteria. Despite showing different killing mechanisms and the absence of any structural homology, microcins have the common characteristic to use Trojan horse strategies to destroy their competitors. In *E. coli*, microcins E492, H47, I47 and M can carry at their C-terminus a siderophore acquired by post-translational modification (61,62).

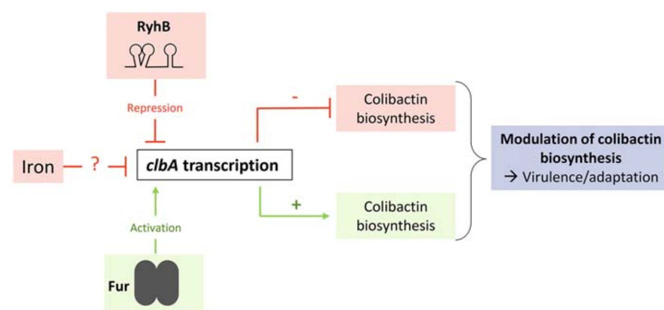

FIG 6 *The* clbA *transcription is regulated by both the ferric uptake regulator (Fur) and the small regulatory noncoding RNA RyhB, leading to the regulation of colibactin production (according to* 53*).*

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There is an intimate link between microcins and iron. Many microcins are induced under conditions of iron limitation and many exploit receptors on sensitive cells that are involved in iron acquisition. To cross the outer membrane of target bacteria, siderophore-microcins indeed hijack siderophore receptors located on target bacteria. Fur binding sites were identified in the promoter region of genes coding for enzymes involved in the biosynthesis of microcins and were found to bind Fur (63). Under restricted iron supply, microcins would be made that recognize the highly induced siderophore receptors on the surface of the target bacteria. Interestingly, up to 85 % of the *E. coli* strains positive for microcins H47 and M are members of group B2 in collections of fecal isolates from humans (64,65). Attempts to accelerate the uptake of antibiotics into Gram-negative bacteria by conjugating siderophore moieties, mimetic of natural siderophoric functionalities, with several different classes of antibiotics constitute a field of research aiming at providing new antibiotics for the treatment of infections (66).

Concluding Remarks

Genetic analysis of different *E. coli* strains showed that those of the phylogenetic group B2 have developed a greater ability to acquire iron (heme receptor and numerous siderophores), to produce the genotoxin colibactin and siderophore-microcins. It is amazing to observe an increase of these *E. coli* strains over the last 30 years in the intestinal microbiota in industrialized countries (67),68. Integrating the regulation of fitness/virulence factors, such as siderophores, colibactin and siderophore-microcins into networks that respond to specific environmental signals, such as the local iron concentration, could result in an accurate production of specific fitness/virulence factors, so that the *E. coli* can adapt to the competitive environment that is the gut and/or the blood.

Iron deficiency is common in infancy, even in industrialized countries. Usual strategies for anemia correction are iron supplementation and iron fortification of foods. The long-term consequences and risks associated with high iron supply in the light of this iron-dependent network described in this review could explain at least in part the increased prevalence of *E. coli* B2, which synthesize a complex repertoire of siderophores and microcins, in the gut of people in industrialized countries.

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