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MINIREVIEW

# $C_4$-Dicarboxylates as Growth Substrates and Signaling Molecules for Commensal and Pathogenic Enteric Bacteria in Mammalian Intestine

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**ABSTRACT** The $C_4$-dicarboxylates (C4-DC) L-aspartate and L-malate have been identified as playing an important role in the colonization of mammalian intestine by enteric bacteria, such as *Escherichia coli* and *Salmonella enterica* serovar Typhimurium, and succinate as a signaling molecule for host-enteric bacterium interaction. Thus, endogenous and exogenous fumarate respiration and related functions are required for efficient initial growth of the bacteria. L-Aspartate represents a major substrate for fumarate respiration in the intestine and a high-quality substrate for nitrogen assimilation. During nitrogen assimilation, DcuA catalyzes an L-aspartate/fumarate antiport and serves as a nitrogen shuttle for the net uptake of ammonium only, whereas DcuB acts as a redox shuttle that catalyzes the L-malate/succinate antiport during fumarate respiration. The C4-DC two-component system DcuS-DcuR is active in the intestine and responds to intestinal C4-DC levels. Moreover, in macrophages and in mice, succinate is a signal that promotes virulence and survival of *S.* Typhimurium and pathogenic *E. coli*. On the other hand, intestinal succinate is an important signaling molecule for the host and activates response and protective programs. Therefore, C4-DCs play a major role in supporting colonization of enteric bacteria and as signaling molecules for the adaptation of host physiology.

**KEYWORDS** $C_4$-dicarboxylates, L-aspartate, succinate, fumarate respiration, nitrogen assimilation, *Escherichia coli*, *Salmonella* Typhimurium, intestine colonization, initial growth, Salmonella

*Escherichia coli* and *Salmonella enterica* serovar Typhimurium belong to the *Enterobacteriaceae*, which are characterized by their metabolic versatility. Hexoses represent the preferred carbon source of enterobacteria and are degraded by glycolysis (1) followed by complete oxidation in the citric acid cycle under aerobic conditions, or by mixed acid fermentation under anaerobic conditions (2–4). $C_4$-dicarboxylic acids (C4-DCs) such as succinate, L-malate, L-tartrate, and L-aspartate represent alternative substrates for growth (5–9). C4-DCs are degraded by the citric acid cycle in combination with the pyruvate bypass under aerobic conditions and by fumarate respiration under anaerobic conditions. C4-DC degradation is subject to glucose repression, and central genes for the degradation of C4-DC substrates are induced by the C4-DC two-component system DcuS-DcuR (10). Major targets of regulation by DcuS-DcuR are the genes encoding the aerobic C4-DC transporter DctA, and of the anaerobic fumarate respiration, namely, the C4-DC/succinate antiporter DcuB, fumarate reductase FrdABCD, and fumarase FumB (10, 11). Fumarate respiration represents an important mode of energy conservation during anaerobic growth of enteric and proteobacteria (12–14).

The physiology, biochemistry, and regulation of hexose and C4-DC metabolism have been studied in detail for enteric bacteria, particularly under defined (laboratory) conditions. The significance of hexoses and hexose derivatives was confirmed for growth in the intestine of mice or the mucus covering the cecal epithelium by commensal and

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pathogenic *E. coli* (15, 16). On the other hand, the role of C4-DCs for the enteric bacteria in the intestine has remained uncertain. Under anaerobic conditions in the intestine, fumarate respiration has been shown to be important for fitness and initial growth of commensal *E. coli* and the enteropathogenic *S.* Typhimurium (17–20). The levels of fumarate in the cecum and intestinal lumen were negligible, whereas those for L-aspartate and L-malate are notable and reach levels that are sufficient to induce DcuS-DcuR dependent genes of fumarate respiration (19, 20). Therefore, C4-DCs represented by L-aspartate and L-malate have an important role for microbiota by driving fumarate respiration (19–22). Under the oxidative conditions in an inflamed intestine, *S.* Typhimurium also oxidizes succinate (and other C4-DCs) by an oxidative central metabolism (23). C4-DCs also serve as a carbon source during aerobic and anaerobic growth of *E. coli* and *S.* Typhimurium (8, 9, 24). L-Aspartate is used as an important nitrogen source (20, 21). The key enzyme of the assimilatory pathway, aspartase AspA, is subject to regulation by the general nitrogen regulatory system (22).

Recent findings suggest that, in addition to its role as substrates, succinate coordinates reactions related to virulence and survival of *S.* Typhimurium in macrophages and in mice (25). Moreover, succinate is an important signaling molecule for the host. Accumulation of microbiota-derived succinate affects transcriptional and posttranslational modifications in the host and activates inflammatory programs, epigenetic regulation, and ROS production (26–28).

Overall, C4-DCs have a major and specific role in the interaction of the mammalian host with enteric bacteria. L-Aspartate and L-malate are important for gut colonization of *E. coli* and *S.* Typhimurium due to their role in fumarate respiration, nitrogen assimilation, and regulation of virulence. On the other hand, succinate produced by the microbiota represents a substrate and signaling molecule for host luminal cells and macrophages. This review will discuss the role of C4-DCs in the growth and colonization of bacteria in the intestine with an overview of recent aspects of succinate as a signaling molecule in host-microbiota interaction and physiology.

## L-ASPARTATE AND L-MALATE AS SUBSTRATES FOR FUMARATE RESPIRATION IN THE INTESTINE

*E. coli* and other enteric bacteria perform fumarate respiration in the absence of electron acceptors like $O_2$ or nitrate (2, 12, 14, 29). Fumarate reductase FrdABCD is membrane-integral and perceives the electrons for fumarate reduction from menaquinol (30). During fermentation of hexoses and other carbohydrates, menaquinone (MK) is reduced at the expense of NADH, formate, or $H_2$ originating from fermentation (1, 3, 31). NADH and $H_2$ are oxidized by NADH dehydrogenase I (NuoA-N) and respiratory hydrogenase (mostly hydrogenase 2 or Hyb in *E. coli* and *S.* Typhimurium) to reduce MK. NuoA-N and Hyb conserve the redox energy in $H^+$ potential (32–35).

*E. coli* ferments hexoses to ethanol, acetate, and formate (or $H_2 + CO_2$) as the main products; additionally, 0.11 to 0.29 mol of succinate are formed per mol of glucose (36, 37). Succinate is the product of endogenous fumarate respiration; the fumarate for this reaction is derived from the phospho-*enol*-pyruvate of glycolysis by carboxylation and the reductive branch of the (anaerobic) citric acid cycle (Fig. 1) (38). Succinate is excreted by transporter DcuC (39, 40). *E. coli* also utilizes exogenous C4-DCs such as fumarate in the medium as the electron acceptor for fumarate respiration (8, 12, 31). The electrons for fumarate respiration are derived mostly from NADH or $H_2$ that originate from glucose fermentation, or glycerol-3P (from glycerol) and $H_2$ as exogenous electron donors (3, 8, 31) (Fig. 1). During fumarate respiration, uptake of external C4-DCs is catalyzed by the antiporters DcuA or DcuB in antiport against succinate (Fig. 1). In addition to fumarate, L-malate, L-aspartate, L-tartrate, and citrate can be utilized by *E. coli* as precursors of fumarate for fumarate respiration. L-Malate, L-tartrate, and citrate are found in significant amounts in fruit or plant materials, while L-aspartate is derived from proteins. Fumarate/succinate and L-malate/succinate antiport is performed preferentially by DcuB, and L-aspartate/succinate antiport by DcuA (8, 21, 39, 40). At high concentrations of fumarate, L-malate, and L-aspartate ($>$0.1 mM), the transporters are mutually active. In addition, DcuC, which mainly functions in succinate

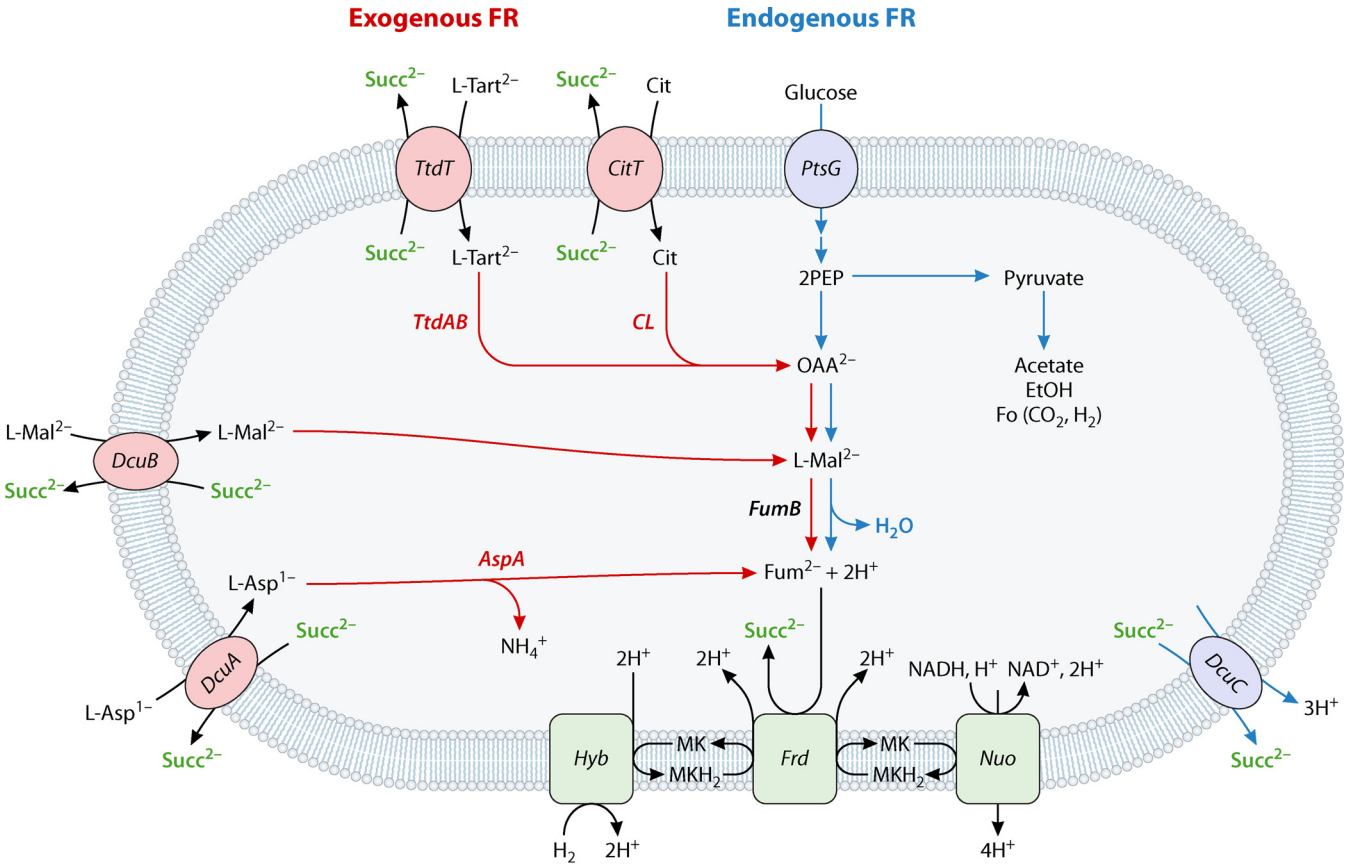


**FIG 1** Exogenous and endogenous fumarate respiration (FR) by *E. coli*. For endogenous FR (fumarate produced during hexose fermentation) up to 15% of the PEP formed during hexose fermentation (37) is carboxylated to yield OAA, which is then converted by the reductive branch of the anaerobic citric acid cycle to succinate. For exogenous FR, L-aspartate, L-malate, fumarate, L-tartrate, or citrate are taken up by antiporters from the medium, and succinate is excreted in an electroneutral antiport. Enzymes and feeding reactions for fumarate formation are shown in blue and red, respectively, joint reactions in green. Details are described in the text and in reviews (3, 8, 31, 35). At concentrations > 0.1 mM fumarate, L-aspartate or L-malate, the transporters DcuA, DcuB, and DcuC are able to replace each other. DcuA, DcuB, and DcuC are present in *S.* Typhimurium as well (19), whereas citrate and tartrate are used only by *E. coli* (6, 46), but not by *S.* Typhimurium for FR (47, 48, 100). AspA, aspartase; CitT, citrate/succinate antiporter; CL, citrate lyase; DcuA, C4-DC antiporter DcuA; DcuB, C4-DC antiporter DcuB; DcuC, C4-DC transporter DcuC; FR, fumarate respiration; Frd, fumarate reductase FrdABCD; FumB, fumarase B; Hyb, hydrogenase; Nuo, NuoA-N; PtsG, glucose transporter of the phosphotransferase system; TtdAB, tartrate dehydratase; TtdT, L-tartrate/succinate antiporter; MK, menaquinone; $MKH_2$, menaquinol.

excretion during fermentation, can substitute for DcuA and DcuB (41, 42). The feeding reactions for fumarate formation from the latter C4-DCs are shown in Fig. 1. After uptake, L-malate and L-aspartate are converted to fumarate by fumarase FumB and aspartase AspA. It appears that FumB and AspA are organized in metabolons with the transporters for efficient channeling and supply of fumarate for respiration (Fig. 2) (43). L-Tartrate and citrate are transported by TtdT and CitT, respectively, in antiport against succinate (6, 44–47). L-Tartrate is dehydrated to oxaloacetate by L-tartrate dehydratase TtdAB, while citrate is cleaved by citrate lyase CL, also producing oxaloacetate (OAA). The OAA is converted to fumarate in *E. coli* by the anaerobic reductive part of the tricarboxylic acid (TCA) cycle and used for fumarate respiration (Fig. 1) (6, 8, 45), while in *S.* Typhimurium, the OAA is decarboxylated by the $Na^+$-translocating OAA decarboxylase into pyruvate (48, 49).

*E. coli* or *S.* Typhimurium deficient in *frdA*, *dcuSR*, *dcuB*, and *aspA* are severely impaired in their capacity to colonize mouse intestine. The most pronounced effects are observed in *frdA* deficiency (18–20), followed by the loss of *dcuS dcuR*, while the effects of *dcuB* and *aspA* deletion are more moderate. Also, the mRNA levels of not only *frdA* but also of *aspA*, *dcuB*, and *dcuC* are greatly increased in the cecum and colon of mice in a DcuR-dependent manner (20). The expression of *dcuA*, on the other hand, was constant, as previously shown *in vitro* (50). In the presence of *E. coli*, *S.* Typhimurium has reduced initial growth in the gut lumen, which is not the case for *dcuAB*- and *frdA*-deficient *E. coli* strains, suggesting that

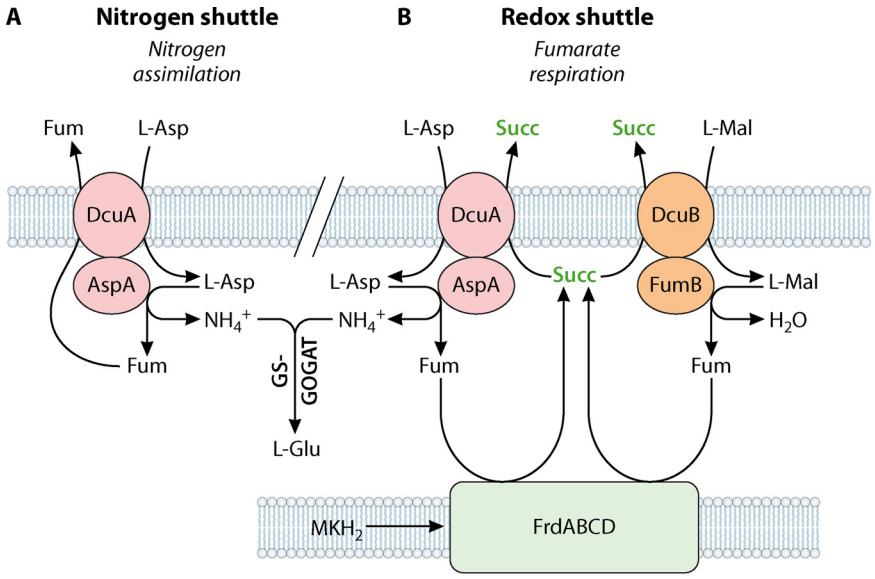


**FIG 2** Scheme for the DcuA/AspA, DcuB/AspA, and DcuB/FumB metabolons of *E. coli*. Complex formation between AspA and FumB with the Dcu transporters is based on interaction studies (43), suggesting metabolon formation and metabolic channeling. The L-aspartate/fumarate antiport used during nitrogen assimilation by DcuA results in net uptake of ammonium ("nitrogen or ammonium shuttle"), the fumarate/succinate or L-malate/succinate antiport in the net uptake of 2 [H] ("H or redox shuttle") for the sake of fumarate respiration. Figure modified from Schubert and Unden (43). AspA, aspartase; DcuA, C4-DC transporter; DcuB, C4-DC transporter; FrdABCD, fumarate reductase; GS-GOGAT, glutamine synthetase (GS)-glutamine 2-oxoglutarate aminotransferase (GOGAT) pathway; Fum, fumarate; FumB, fumarase B; L-Asp, L-aspartate; L-Mal, L-malate; $MKH_2$, menaquinol; Succ, succinate.

fumarate respiration promotes colonization of *E. coli* and establishes colonization resistance against *S.* Typhimurium (19). Therefore, fumarate respiratory genes, including the regulatory genes *dcuS* and *dcuR*, are important for gut colonization. Remarkably, *frdA* and *dcuS dcuR*, central for both exogenous and endogenous fumarate respiration, were most important for gut colonization; *dcuB* and *dcuA*, required for only externally supplied C4-DCs, were less significant for colonization efficiency but support initial growth (19, 20). The hydrogenase Hyb, the major uptake hydrogenase for MK reduction, is also essential for intestinal colonization of *S.* Typhimurium and *E. coli* (51, 52). The $H_2$ could originate from formate that is converted by *E. coli* formate-hydrogen lyase to $H_2$ and $CO_2$, or from the fermentative metabolism of gut microbiota. In an inflamed gut, *S.* Typhimurium utilizes formate directly as an electron donor for aerobic and nitrate respiration. The reaction involves formate dehydrogenases of aerobic (*fdo* genes) and (*fdn* genes) nitrate respiration, as concluded from the decreased fitness of the corresponding mutants (53). Taken together, the data demonstrate the significance of fumarate respiration for colonization, and in particular of the genes that are also required for endogenous fumarate respiration. The *frdA* is expressed almost constitutively under anaerobic conditions in the interest of endogenous fumarate respiration and is stimulated by external C4-DCs by a factor of 1.5 to 2 only (10, 20), in contrast to the stimulation of *dcuB* by a factor of 5.6 to 11.6 (10, 20).

The significance of C4-DCs and of fumarate respiration for establishing growth in the intestine appears to be mostly related to its capacity to facilitate redox balancing in hexose fermentation. Thus, endogenous fumarate respiration provides an alternative means to consume reducing equivalents during fermentation, such as NADH or $H_2$. Therefore, endogenous (and exogenous) fumarate respiration allows metabolic flexibility and the production of alternative substrates.

The lumen of the murine small intestine contains significant levels of L-aspartate (>1 mmol/kg wet mass), depending on diet and mouse breeding (19, 20), and the contents in the cecum are still notable (≥0.1 to 1 mmol/kg wet mass). In addition, L-aspartate-related compounds, such as L-asparagine or fructose-asparagine (Fruc-Asn) (54), can

provide L-aspartate in the intestine. The contents of L-malate were lower but also significant, whereas those for fumarate were negligible (19, 20).

L-Aspartate and L-malate levels in the intestine exceed the $K_m$ values for uptake by DcuA and DcuB (43 and 110 $\mu$M, respectively) (21, 40), suggesting efficient uptake of C4-DCs by *E. coli*. The C4-DC-dependent stimulation of DcuS-DcuR occurs via the periplasmic sensor domain of DcuS (55, 56). The apparent $K_m$ for the activation of DcuS by C4-DCs is in the range of 0.5 to 3 mM (57). Schubert and coworkers confirmed that C4-DC concentrations of the small intestine or the cecum induce the expression of a *dcuB-lacZ* reporter fusion, which is in agreement with the high levels of mRNA of fumarate respiratory genes in mouse intestine (20). Most stimulation will be caused by L-aspartate, which stimulates DcuS with an apparent $K_m$ of 2 mM (57) and L-malate. In summary, endogenous and exogenous fumarate respiration contribute to initial growth of *E. coli* and *S.* Typhimurium in mouse intestine. L-Aspartate and, to a lesser extent, L-malate are the main substrates for fumarate respiration by external C4-DCs. Remarkably, L-aspartate is also a major regulator of chemotaxis in *E. coli* utilizing the chemoreceptor Tar for perception (58).

The mid small intestine and colon of mice are microaerobic with approximately 7.8 mbar $O_2$ (59), which allows an almost half-maximal expression of FNR-regulated fumarate respiration (60, 61). Under the same $O_2$ tension, the microaerobic oxidase encoded by *cydAB* is strongly expressed (61, 62), and the microaerobic conditions in the intestine are compatible with the concurrent expression of the genes for fumarate and microaerobic respiration (17–20). Fumarate and microaerobic respiration therefore coexist in the homeostatic mouse intestine. Under inflammatory conditions, reactive nitrogen species (RNS) and oxygen species (ROS) are formed (63, 64). RNS generate nitrate ($NO_3^-$), an important electron acceptor for facultative anaerobic bacteria (65). Nitrate represses fumarate respiration and *frdABCD* (66), indicating a decreased role for C4-DC utilization under inflammation (53). In support of this speculation, DcuS-DcuR is dispensable for *E. coli* fitness under inflammatory conditions (20).

## L-ASPARTATE AS A HIGH-QUALITY NITROGEN SOURCE

L-Aspartate is a high-quality nitrogen source and is capable of saturating the nitrogen demand of *E. coli* under aerobic and anaerobic conditions (20–22). Nitrogen assimilation from L-aspartate requires the transporter DcuA and aspartate ammonia lyase AspA (Fig. 2). L-Asparagine (L-Asn) is deamidated to L-aspartate in the periplasmic space of *E. coli* by asparaginase AnsB (67, 68), and then utilized in the same way as L-aspartate. Fruc-Asn, a primary nutrient of *S.* Typhimurium in an inflamed intestine (54), is also an excellent source of nitrogen and carbon. Fruc-Asn is deamidated in the periplasmic space by FraE, transported into the bacterial cell by FraA, phosphorylated by FraD, and hydrolyzed to glucose-6-P (G6P) and L-aspartate by FraB (69).

AspA catalyzes the deamination of L-aspartate, producing fumarate and ammonium (21, 22). Assimilation of the ammonium occurs by glutamine synthetase GS (or GlnA) and glutamine 2-oxoglutarate aminotransferase GOGAT (or GltBD) yielding L-Glu (70, 71). The DcuA-AspA-GS-GOGAT pathway saturates the nitrogen requirement of *E. coli* efficiently (21, 22).

The expression of *aspA* and *dcuA* is essentially constitutive in *E. coli* (22, 50). AspA is integrated, however, into the nitrogen regulatory system of the central nitrogen regulator GlnB (alternative name PII) (72). GlnB regulates AspA activity in response to nitrogen availability in the cell (22). Under nitrogen-limited conditions, the deaminase activity of AspA and ammonium release is stimulated 2-fold by GlnB when the regulator is activated by uridylylation and binding of ATP and 2-oxoglutarate (Fig. 3). The stimulation is lost in the deuridylylated state of GlnB that prevails under nitrogen-saturated conditions. Overall, GlnB regulates the utilization of L-aspartate to ensure nitrogen supply under nitrogen-limited conditions. The presence of high levels of L-aspartate in mouse (20) or bovine (73) intestines, together with specific regulation, highlights the physiological relevance of L-aspartate as a source of nitrogen in *E. coli*.

The fumarate released by the AspA reaction is excreted in aerobic growth nearly stoichiometrically by DcuA (Fig. 2) when other carbon sources are available (21). Under

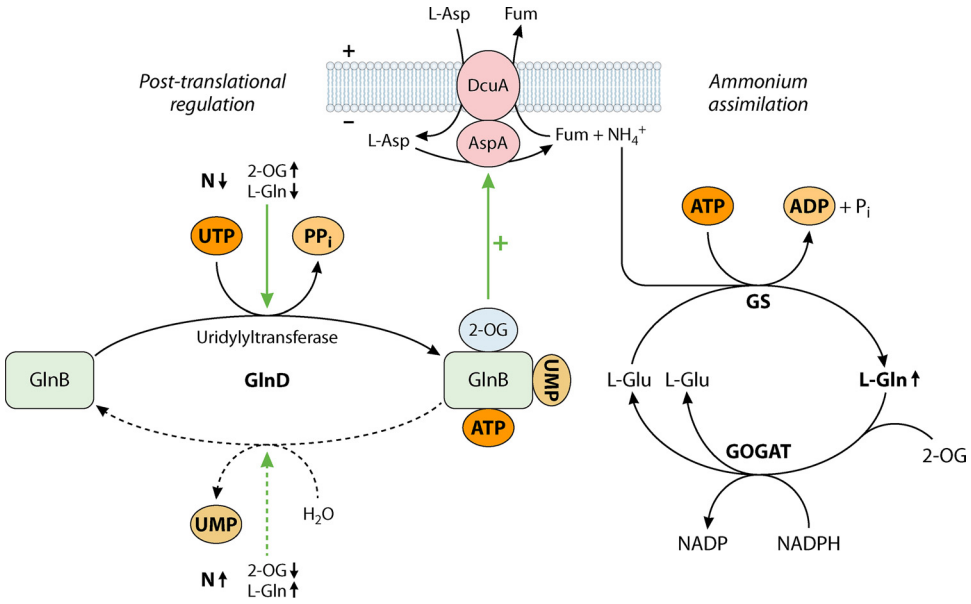


**FIG 3** Ammonium assimilation from L-aspartate using DcuA-AspA for uptake and intracellular ammonium release, ammonium assimilation by GS-GOGAT, and the GlnB regulatory system. The scheme shows the uptake of L-aspartate by DcuA and ammonium release by the DcuA-AspA metabolon, the ammonium assimilation via the common GS-GOGAT pathway yielding L-Glu, and the regulation of AspA by the GlnB regulatory system and regulatory factors. N↓, nitrogen-limited conditions; N↑, nitrogen-saturated conditions; 2-OG, 2-oxoglutarate; UTP, uridine-triphosphate; $PP_i$, diphosphate; $P_i$, phosphate; UMP, uridine-monophosphate; GlnD, uridylyltransferase/uridylyl-removing enzyme; Fum, fumarate; PII, nitrogen regulator GlnB; DcuA, aerobic L-aspartate transporter; AspA, aspartate ammonium-lyase; GS, glutamine synthetase GlnA; GOGAT, glutamine 2-oxoglutarate aminotransferase GltBD.

anaerobic conditions, the fumarate is used as a substrate for fumarate respiration and excreted only after reduction to succinate (8, 20, 39). DcuA therefore catalyzes an L-aspartate/fumarate or L-aspartate/succinate substrate/product antiport under aerobic and anaerobic conditions, respectively (Fig. 2). The L-aspartate/fumarate antiport results in the net uptake of ammonium and serves as an ammonium shuttle for the purpose of nitrogen assimilation. The L-aspartate/succinate antiport during fumarate respiration, on the other hand, represents a redox shuttle (in addition to its function as the ammonium shuttle), similar to DcuB catalyzing the fumarate/succinate antiport (Fig. 2B).

## COORDINATION OF L-ASPARTATE AND C4-DC METABOLISM BY C4-DCS, CATABOLITE CONTROL, RESPIRATION, AND AMINO ACID AVAILABILITY

Transcriptional regulation by C4-DCs is the result of direct regulation by the DcuS-DcuR two-component system (8, 10, 11, 74). DcuS-DcuR-regulated genes encode proteins catalyzing uptake and initial catabolic steps of C4-DC catabolism (DctA, DcuB, FumB, and FrdABCD proteins). Most DcuS-DcuR-regulated genes are subject to multiple regulation, including FNR for aerobic regulation, NarX-NarL for nitrate regulation, cAMP-CRP for catabolite control, and Lrp responding to amino acids. Regulation of *frdA*, *dcuB*, and *dctA* by electron acceptors $O_2$, nitrate, and C4-DCs by FNR, NarX-NarL, and DcuS-DcuR has been discussed earlier (10, 11, 50, 66, 75, 76), whereas regulation by cAMP-CRP and Lrp has been analyzed more recently (11, 20, 77). Many proteins that display altered levels in response to fumarate are not members of the DcuS-DcuR regulon but are subject to catabolite regulation by cAMP-CRP (77, 78). This includes proteins of the citric acid cycle and associated pathways, proteins involved in motility and chemotaxis under anaerobic conditions, and oxidative stress (77, 78). Cellular cAMP levels are known to increase during growth on low-quality carbon and energy sources, such as C4-DCs and acetate (79–81), which has been related to the fumarate effect on the expression of genes that are not under direct DcuS-DcuR control (77). Genes regulated by DcuS-DcuR are apparently often under the control of cAMP-CRP or FNR also, as shown for the *dctA* and *dcuB* promoters in Fig. 4. Transcriptional activation by cAMP-CRP and FNR is related to DNA-bending of the promoter regions (82, 83). It has been suggested that regulation by

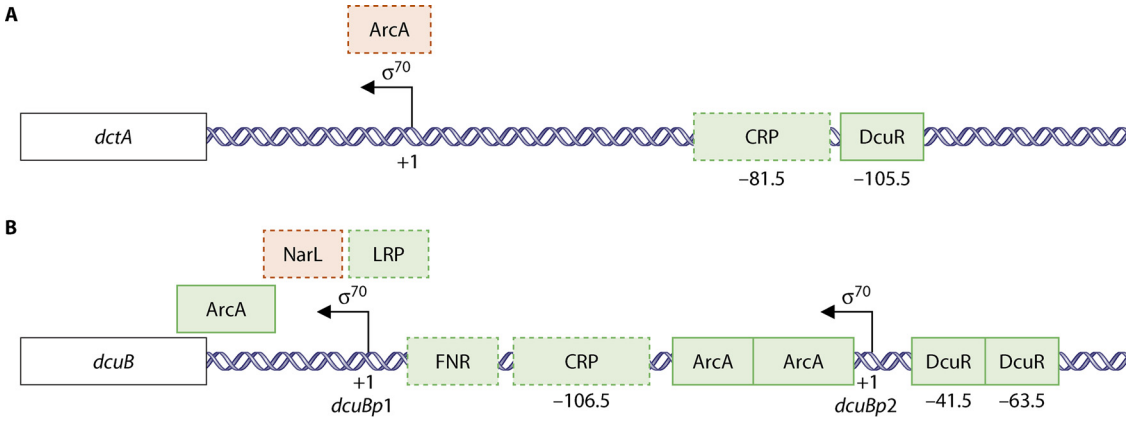


**FIG 4** Promoter regions of *dctA* (A) and *dcuB* (B) and binding sites for transcriptional regulators DcuR, cAMP-CRP, FNR, ArcA, NarL and Lrp. The binding sites have been determined experimentally (solid line) (101–103) or by the presence of consensus sites (broken lines). Transcriptional regulators exerting positive (green) or negative (red) regulation on the promoter are annotated. Numbering gives the position relative to the transcriptional start sites of the promoters. The location of the binding sites for NarL and Lrp at *dcuB* have not been identified.

DcuR requires DNA-bending by cAMP-CRP to induce expression in response to C4-DCs via DcuS-DcuR (81).

Growth on $C_4$-dicarboxylates also requires gluconeogenesis for the synthesis of the glycolytic substrates and cell components derived from these. The gluconeogenic switch is known for the transition from glucose to acetate-grown *E. coli* (84–86). A similar switch and lower growth rates are observed for growth on C4-DCs, with an increase in all TCA cycle enzymes, the pyruvate bypass malic enzyme MaeB, and PEP-carboxykinase PckA (77). In the same way, the enzymes for the degradation of amino acids and fatty acids are increased to feed the TCA cycle (77).

The *dcuB* and *frdA* genes are targets for regulation by the transcriptional regulator Lrp (20), and the promoter regions of *dcuB* (Fig. 4) and *frdA* contain putative Lrp binding sites. Lrp is a global transcriptional regulator that responds to L-leucine and controls the expression of about 10% of *E. coli* genes; it is presumed to function by interaction with other regulators (87, 88). Mammalian intestine is an amino acid-rich environment (20, 73) containing almost all proteinogenic amino acids. The regulation of *dcuB* and *frdA* by Lrp is suggested to coordinate the utilization of L-aspartate as a source for fumarate respiration, for ammonium, and for degradation and feeding into the citric acid cycle.

## SUCCINATE AND DcuB AS TRIGGERS FOR INTRACELLULAR INFECTION AND HOST-BACTERIUM SIGNALING

Intestinal colonization by enteric bacteria is established in two main steps—initial growth and growth in the inflamed intestine. Commensal *E. coli* grows, in contrast to *S.* Typhimurium or pathogenic *E. coli* strains, without initiation of inflammation. Inflammation drastically alters the intestinal environment and causes the release of host-derived electron acceptors, such as oxygen, nitrate, and tetrathionate (17, 64, 89). Electron acceptors promote the blooming of enterobacteria by conveying a growth advantage over the resident microbiota. Under anaerobic conditions in the normal intestine, the TCA cycle is repressed, but the reductive branch leading from oxaloacetate to succinate is active (19, 38), which is important for fumarate respiration and initial growth of *E. coli* and *S.* Typhimurium. The oxidative conditions in an inflamed gut, however, allow expression of the complete TCA cycle, which enables the utilization of the microbiota-derived fermentation product succinate as a carbon source (23). Bacteroides strains are the major succinate producers in the microbiota.

Apart from its benefit for efficient colonization of *S.* Typhimurium in an inflamed intestine, succinate is an activation signal for virulence of *S.* Typhimurium (25). Uptake of host succinate induces *Salmonella* pathogenicity island 2 (SPI-2) and antimicrobial resistance, which is vital for intracellular survival in macrophages. The response

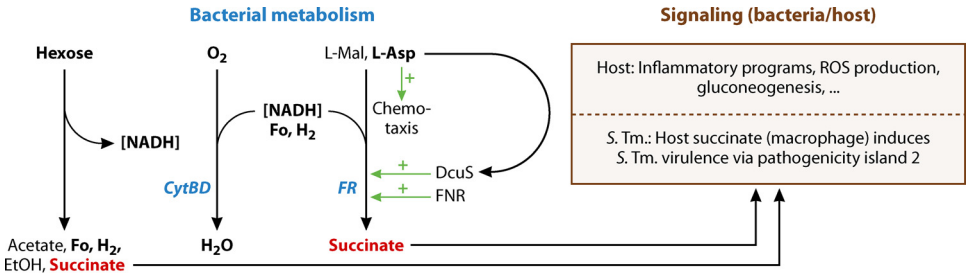


**FIG 5** $C_4$-dicarboxylates as substrates or products of metabolism, and as signaling molecules for host/microbiota interaction in the intestine. Hexose fermentation, fumarate respiration (FR) and microaerobic respiration run in parallel under the microaerobic conditions of the intestine. The intestinal C4-DCs (black) serve as stimuli of the DcuS regulated metabolism of enteric bacteria, and of chemotaxis by Tar. Succinate produced by the enteric bacteria (red) or other microbiota is used for signaling or for communication with host cell, and succinate of host cells (macrophages) stimulates virulence and pathogenicity of *S.* Typhimurium. See the text for details. CytBD, microaerobic Cyt *bd* oxygen reductase; Fo, formate; ROS, reactive oxygen species; other abbreviations as in Fig. 1 to 4.

depends on the presence of DcuB, suggesting that DcuB is involved in the uptake or sensing of succinate (25). SPI-2 is required to translocate effector proteins from vacuolar-resident bacteria into host cells (90). In contrast, SPI-1 is essential for triggering gastrointestinal diseases, whereas it is dispensable for systemic infections (90). Therefore, in addition to the role of C4-DCs as substrates for initial growth of *E. coli* and *S.* Typhimurium in the intestine, succinate appears to function as a trigger inducing survival of the bacteria in the host cell.

Besides its role in bacteria in the activation of virulence factors and as a nutrient for colonization (23, 24), succinate is an important signaling molecule for the host. Accumulation of succinate in the host cytosol affects posttranslational modification by succinylation and activates inflammatory programs, epigenetic regulation, and ROS production (26–28). In addition, microbiota-derived succinate is used for gluconeogenesis (91–93) and thermogenesis (94, 95) by luminal host cells. Moreover, higher levels of circulating succinate have been associated with obesity and gut dysbiosis disorders. The gut microbiota is the predominant producer of luminal succinate (96–99). Understanding the role and control of bacterial succinate production could be a starting point for the development of probiotic interventions to modulate gut-derived succinate and to target obesity-related diseases (95).

## CONCLUSION

Fumarate respiration, whether using endogenously produced fumarate from hexose fermentation or consuming exogenously-supplied C4-DCs, was found to promote initial growth and colonization of the mammalian gut by intestinal bacteria. The significance of C4-DCs contrasts their rather low levels found in the intestine compared to sugars and sugar derivatives. It is suggested that a major role of endogenous and exogenous fumarate respiration is to provide a means for redox balancing under anaerobic conditions (Fig. 5). For the same reason, microaerobic respiration might be important for efficient colonization of the intestine by *E. coli*.

C4-DCs serve as important stimuli for regulating metabolism and physiology of enteric bacteria, using the two-component system DcuS-DcuR and the chemotaxis receptor Tar for perception (Fig. 5). Succinate produced by the microbiota also represents an important signaling molecule for host-microbiota interaction and a nutrient for host cells that may be involved in intestinal dysbiosis disorders (Fig. 5). On the other hand, succinate of host cells, such as macrophages, stimulates virulence and pathogenicity of *S.* Typhimurium. Identifying these roles and functions will open up new avenues for understanding and controlling host-microbiota interaction.

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