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Metabolism and Fitness of Urinary Tract Pathogens

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Introduction

Among common infections, urinary tract infections (UTI) are the most frequently diagnosed urologic disease. The majority of UTIs are caused by *Escherichia coli* and these uropathogenic *E. coli* (UPEC) infections place a significant financial burden on the healthcare system by generating annual costs in excess of two billion dollars (1, 2) in the United States alone.

Overview of Extraintestinal pathogenic *E. coli* (ExPEC) and uropathogenic subset (UPEC)

Escherichia coli, one of the most important model organisms in the laboratory, is the best studied microorganism. The primary niche occupied by *E. coli* is the lower intestinal tract of mammals, where it resides as a beneficial component of the commensal microbiota. Although it is well-known that *E. coli* resides in the human intestine as a harmless commensal, specific strains or pathotypes have the potential to cause a wide spectrum of intestinal and diarrheal diseases. For example, at least six pathotypes have been described: enterohemorragic, enteropathogenic, enterotoxigenic, enteroaggregative, diffuse-adherent, and enteroinvasive *E. coli* (respectively, EHEC, EPEC, ETEC, EAEC, DAEC, and EIEC). On the other hand, extra-intestinal diseases that include urinary tract infection (UTI), bacteremia, septicemia, and meningitis can be caused by additional pathotypes known as extraintestinal pathogenic *E. coli* (ExPEC) (3). The loss or gain of mobile genetic elements is responsible for the ability of *E. coli* to cause a broad range of human diseases (4). The core genome shared by all *E. coli* strains represents approximately 3,200 gene families, while the pan genome that represents the collective gene content for all sequenced *E. coli* strains exceeds 60,000 gene families (5). Thus, for each *E. coli* strain, which contains 4800 genes on average, it is the specific composition of horizontally acquired genetic material that determines its ability to cause a certain disease and be defined as a specific pathotype (6).

Intestinal *E. coli* pathotypes, like the infamous EHEC O157:H7 serotype, resides in the bovine intestine as a commensal bacterium and cause severe diarrheal disease only when accidentally introduced into the human intestinal tract. In contrast, extraintestinal *E. coli* pathotypes reside harmlessly in the human intestinal microenvironment but, upon access to sites outside of the intestine, become a major cause of human morbidity and mortality as a

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consequence of invasive UTI (pyelonephritis, bacteremia, or septicemia) (7, 8). Thus, extraintestinal pathotypes like uropathogenic *E. coli* (UPEC) possess an enhanced ability to cause infection outside of the intestinal tract and colonize the urinary tract, the bloodstream, or cerebrospinal fluid of human hosts (8, 9). It follows that extraintestinal pathogenic *E. coli* possess the unique ability to shift its behavior between harmless colonizer of the nutrient-rich human intestine and virulent pathogen of the nutritionally limited bladder (10–13) (Figure 1). Here, we discuss the current understanding of the role for uropathogenic *E. coli* metabolism and physiology in adapting to these diverse host microenvironments.

Traditional extraintestinal *E. coli* virulence factors

Studies in uropathogenesis are largely focused on pathogen-specific virulence properties including toxins, adhesins, secretion, motility, and iron acquisition systems, and mechanisms to avoid the innate and adaptive immune response. Epidemiological studies have identified a number of specific virulence factors or genetic determinants associated with extraintestinal pathogenic *E. coli* (ExPEC) isolates. Extraintestinal virulence genes like those encoding P-fimbriae or hemolysin are frequently clustered in genomic islands known as pathogenicity-associated islands (PAIs) (14) and encode a variety of fimbrial and non-fimbrial adhesins, toxins, and iron acquisition systems (7, 15). Of *E. coli* pathotypes, ExPEC isolates generally have the largest number of PAIs and horizontally acquired genes; specifically, the prototype pyelonephritis UPEC strain CFT073 has 13 PAIs and is the largest genome (5,388 predicted genes) of sequenced *E. coli* strains (4). One outstanding question, however, is whether these horizontally acquired determinants are maintained in UPEC because they confer an advantage during intestinal colonization or are selected for increased extraintestinal fitness (16, 17).

An intriguing aspect of the pathogenesis of *E. coli* UTI is the lack of a single dominant virulence factor or common set of virulence determinants shared by all UPEC strains but absent from commensal *E. coli* or intestinal pathogens. Bacterial metabolism during infection has only recently been appreciated to contribute to persistence as much as their virulence properties. Due to the requirement for these *E. coli* to replicate in and colonize both the intestine and extraintestinal environments, we posit that physiology and metabolism of ExPEC strains is paramount. Indeed, we propose that the ability to survive in the urinary tract depends as much on bacterial physiology and metabolism as it does on the well-considered virulence determinants (18).

From the intestine to the urinary tract

Studies of ExPEC or UPEC growth in the intestine are limited, however, we know *E. coli* strains reside and grow in the nutrient-rich mucus lining of the mammalian intestine (19, 20). Colonization studies with other *E. coli* have shown the Entner Duodoroff pathway, and gluconate or other sugar acids, are required for intestinal growth of commensal *E. coli* (21). *E. coli* acquires nutrients from the intestinal mucus, a complex mixture of glycoconjugates, and up-regulates genes that encode enzymes involved in the catabolism of N-acetylglucosamine, sialic acid, glucosamine, gluconate, arabinose and fucose (21, 22). EHEC O157:H7 requires the same central carbon pathways as do commensal strains, and mutations in pathways that utilize galactose, hexuronates, mannose, and ribose resulted in

colonization defects (22). Further, multiple mutations in a single strain had an additive effect on colonization levels suggesting that some *E. coli* strains depend on the simultaneous metabolism of up to six sugars to support colonization of the intestine (22). These findings suggest that *E. coli* uses multiple limiting sugars for growth in the intestine and supports the assertion that *E. coli* grows in the intestine using simple sugars released upon breakdown of complex polysaccharides by anaerobic gut residents (23, 24).

Synthesis and degradation of glycogen, an endogenous glucose polymer, plays an important role for *E. coli* during colonization of the mouse intestine by functioning as an internal carbon source for the bacterium during nutrient limitation (25, 26). When faced with limiting sugars due to consumption by other colonizing bacteria, *E. coli* can also switch from glycolytic to gluconeogenic substrates to sustain growth in the intestine (27). For example, EHEC utilizes glycolytic substrates and switches to gluconeogenic substrates when present in the intestine with commensal *E. coli* that are utilizing glycolytic pathways for *in vivo* growth (27). That competition *in vivo* can alter preferred routes of carbon flux through the central pathways, introduces the notion that metabolic flexibility and increased capacity to utilize diverse carbon sources are likely important for successful long-term intestinal colonization by extraintestinal *E. coli* (20).

Commensal *E. coli* that are resident in the intestine are three times more likely to belong to the phylogenetic group B2 (ExPEC primarily belong in B2) than transient intestinal colonizers (28, 29). If ExPEC strains are superior colonizers of the human intestine then it is expected that horizontally acquired genomic islands and PAIs must be contributing to enhanced persistence in the intestine. Recent studies have shown that acquisition and regulation of genes that encode proteins to transport and catabolize prebiotic fructooligosaccharides provides a fitness advantage in the intestine for ExPEC strains (30, 31). The gene cluster required for ExPEC growth on fructo-oligosaccharides encodes two glycoside hydrolases that belong to family 32 in the carbohydrate active enzyme database (CAZY) and a predicted cytoplasmic fructokinase (30). The latter is important because fructose must be phosphorylated to be catabolized by *E. coli*. Another horizontally acquired gene cluster proposed to provide a fitness advantage in the intestine has been discovered in ExPEC, the *frz* operon, which also encodes a sugar kinase in addition to two aldolases, and IIA, IIB, and IIC fructose family PTS transporter subunits (32). These findings suggest that ExPEC genomic islands and PAIs may indeed provide added metabolic flexibility and promote persistence in the competitive intestinal microenvironment.

Models for studying UTI

There are a number of models that have been successfully used to study uropathogenesis and identify virulence and fitness factors. The two primary models discussed here are the murine model of ascending infection (33) and bacterial cultivation in human urine *ex vivo*. For the latter, urine is collected and pooled from healthy donors that have not been exposed to antibiotics. The pooled urine can be filter sterilized and used as a growth medium (34–36). Artificial urine has also been used as a growth medium (37). It is notable that growth in urine *ex vivo* is not a uropathogenesis trait *per se* as a number of non-pathogenic bacteria are capable of growth in urine (34, 38). Unlike growth in urine, the ascending model of UTI is

capable of differentiating non-pathogenic *E. coli* from uropathogenic isolates. Only UPEC or ExPEC strains are able to successfully colonize the bladder and kidneys of mice in this model. It is notable that the ascending model can be used with a number of inbred mice; CBA/J, C3H, and C57BL/6 have been used to study experimental UTI.

Experimental assessment of gene expression

Cultivation of uropathogenic *E. coli* in human urine has been useful to identify genes and proteins that are differentially expressed in urine as compared to during growth in LB or defined medium. The largest group of uniformly upregulated genes and proteins are those involved in iron acquisition (34, 39, 40). Microarray experiments comparing transcription of genes between urine and LB identified numerous genes that are upregulated during growth in human urine. Among those functions that are highly upregulated in urine are; sialic acid transport and catabolism, siderophore biosynthesis and uptake, arginine and branched-chain amino acid transport, histidine transport, serine metabolism, nitrate and formate respiration, mannonate catabolism, and galactoside transport (39). Similar experiments have also been performed with asymptomatic bacteriuria *E. coli* strain 83972 and compared gene expression between human urine and MOPS defined medium. In that work it was found that arginine, methionine, valine, uracil, adenine, and isoleucine are limiting nutrients in human urine and are essential for efficient growth of *E. coli* 83972 in urine (41).

Proteomic analysis of UPEC during growth in human urine has produced complementary findings. The outer membrane proteome is highly enriched for TonB-dependent receptors for siderophores and other iron-containing compounds (34). Outer membrane lipoproteins that are produced in human urine are D-methionine binding and uptake, a pectin methylesterase, and a uroporphyrin methyltransferase. This study also found increased production of BtuB, the cobalamin (B12) receptor and Tsx, which is a nucleoside-specific transport protein (34). Soluble proteins that are upregulated in human urine include many periplasmic substrate-binding proteins. These include DppA and OppA, involved in peptide transport, LivK that binds leucine, and HisJ involved in histine uptake (18). Similar to what was found by microarray, proteomic analysis also identified upregulated proteins involved with sialic acid catabolism and transport, mannonate metabolism, and serine and arginine biosynthesis (18). Overall the findings from using human urine as a model for UPEC has revealed that this milieu is iron-limited, contains some amino acids but is limited for branched chain amino acids, and appears to induce a profile of carbon metabolism consistent with scavenging mucosal sugars.

Description of the urinary tract host niche

In contrast to the nutritionally diverse intestine, urine in the bladder is a high-osmolarity, moderately oxygenated, iron-limited environment that contains mostly amino acids and small peptides (34, 37, 39, 42). It is therefore not surprising that defects in the both branches of the pentose phosphate pathway, the Entner-Doudoroff pathway, and glycolysis have limited or no impact on *E. coli* fitness in the bladder and kidney microenvironments (18, 43). Studies on UPEC metabolism during UTI have revealed that the ability to catabolize the amino acid D-serine in urine, which is both a nutrient and a signaling mechanism to trigger

virulence, supports UPEC growth in the nitrogen-rich urinary tract (44). The utilization of short peptides and amino acids as a carbon source during bacterial infection of the bladder and kidneys is also supported by the observation that UPEC mutants defective in peptide import have reduced fitness during UTI while auxotrophic strains do not (18, 43). Metabolism of nucleobases is also required for *E. coli* colonization of the bladder. Signature-tagged mutagenesis screening identified a mutant in the dihydroorotate dehydrogenase gene *pyrD* (45) and in a separate transposon screen, a gene involved in guanine biosynthesis, *guaA*, was also identified; a *guaA* mutant was found to be attenuated *in vivo* during UTI (36). Both are supported by the recent finding that *E. coli* are rapidly replicating in the bladder (46, 47).

The host urinary tract niche has also been defined by using transcriptome analysis of bacteria stabilized immediately in the urine from patients experiencing acute UTI. These studies have identified *E. coli* genes that are upregulated during human infection. It is notable that there are many upregulated genes that are also upregulated in human urine, for example iron acquisition genes are highly expressed in humans and in urine *ex vixo*; however, there are also many genes that are upregulated during UTI that are not upregulated in human urine, *e.g.* host-specific genes (46–48). Many of the host-specific UPEC genes are involved in metabolic processes. Entire gene clusters for ethanolamine and phosphonate metabolism were upregulated by UPEC isolated directly from human bladders (48). Genes encoding proteins for sulfate/thiosulfate uptake, taurine uptake, and alkane sulfonate uptake were identified as host-specific induced genes. This study also found that potassium import and nickel import are also highly upregulated processes during UTI. Consistent with increased demand for nickel was also the observed upregulation of genes that encode nickel-containing metallo-enzymes, such as formate hydrogen lyase N and hydrogenase (47, 48). RNAseq analysis of bacteria directly from UTI also identified upregulation of genes encoding ATP synthase, mannose-specific PTS components, carbamoyl-phosphate synthase, pyruvate dehydrogenase, nitrate reductase, ribonucleotide reductase, branched-chain amino acid uptake, and methionine biosynthesis (47).

Iron acquisition

There are three classes of iron-uptake systems: siderophores, hemophores (or heme-binding and uptake systems), and direct ferrous iron (FeII) uptake systems (such as ferric citrate uptake) in UPEC. Siderophores are small molecules that are secreted into the environment, have very high affinity for ferric iron, and can strip the metal ion from other complexes within the host, or bind rare free ferric iron. Once a siderophore binds iron, it may be bound by specific outer membrane receptors. Hemophores, on the other hand, are small secreted proteins that bind heme with high affinity before being imported back into the bacterium through specific importers (49). Finally, *E. coli* contains conserved ABC transporters, encoded by the *feo* operon, capable of directly importing ferrous iron (50).

Both siderophore- and hemophore-mediated iron uptake depend on ferric iron. TonB is an inner membrane protein also necessary for all ferric iron uptake receptors in *E. coli*. TonB mutants are defective for colonizing kidneys in coinfection in a mouse model of UTI, a defect that was complementable (51). Additionally, in independent challenge the *tonB*

mutant strain caused UTI at reduced CFU and reduced kidney colonization compared to the parental and complemented strains at 48 HPI. The prototype UPEC strain CFT073 contains at least ten ferric uptake systems and several putative systems (52). Mutations that disrupt salmochelin, enterobactin, heme and other siderophores are outcompeted by wildtype UPEC in coinfection (53–57)).

Iron acquisition appears to be especially important in UTI. The iron-chelating hydroxamate siderophore aerobactin is more common among UPEC strains than in fecal/commensal *E. coli* (58, 59) and the presence of at least one siderophore appears to be a common feature of UPEC strains (60). Gene expression studies in urine from patients suffering from bacterial UTI using microarray suggests that bacterial iron acquisition systems are up-regulated during infection (46). Isotope dilution studies of siderophore activity, which can be more sensitive than transcript/expression analysis, confirm that siderophore activity is increased in UPEC compared to commensal strains of *E. coli* (61). Highlighting their importance, there is substantial notable redundancy in iron acquisition systems in UPEC. Aerobactin-, enterobactin- and heme-mediated iron uptake can each complement the activity of one another. Isogenic mutants of aerobactin or enterobactin were shown to be no different from wildtype in kidney colonization in a mouse model of UTI (51). Vaccine studies using siderophore receptors or heme binding proteins as antigens and a mucosal route of delivery show protection from transurethral challenge in the mouse model of UTI (42), offering further support that iron systems are requisite virulence factors during the course of a UTI.

Amino acid and peptide transport and catabolism

UPEC growing in human urine induces expression of multiple isoforms of both dipeptide- and oligopeptide-binding proteins, both of which were found to be required for UPEC to effectively colonize the bladder and kidneys (18). Since the nutrient content in the kidney is expected to be very different from urine in the bladder, it is surprising that mutants lacking the ability to produce peptide-transport proteins were attenuated in both the kidneys and bladder because growth in urine mainly mimics only the bladder microenvironment. The host renal physiology might be expected to provide UPEC with several readily metabolized carbon sources during reabsorption of the kidney glomerular filtrate in the tubules; however, the ischemic damage caused by UPEC during pyelonephritis could alter nutrient availability (62). Lack of a fitness defect for UPEC amino acid auxotrophs during bladder and kidney colonization and impaired colonization of bladder and kidneys for peptide-transport deficient mutants indicates that these bacteria actively import short peptides found in urine and suggests that peptides or amino acids represent the primary carbon source for *E. coli* during UTI (18). In fact, dissimilatory acetate metabolism coupled to the degradation of amino acids during *E. coli* colonization of the bladder and kidneys shows that ExPEC are adapted to acetogenic growth rather than acetate assimilation (63). Further, prolonged asymptomatic carriage of *E. coli* in the bladder selects for mutations that increase expression of D-serine deaminase and peptide/amino acid transport in *E. coli* (64). Gluconeogenic amino acids, like D- and L-serine, can be degraded to oxaloacetate or to pyruvate that can enter the TCA cycle, which is necessary to provide substrates for gluconeogenesis when *E. coli* use amino acids as a carbon source. Consistent with peptides and amino acids being important carbon sources during UTI, only bacteria with defects in peptide transport,

gluconeogenesis, or the TCA cycle demonstrate a fitness defect during colonization of the host urinary tract (Figure 2) (18, 43, 65).

Central carbon pathways

Transcriptome and proteomic studies have been useful to identify iron acquisition and many nutrient transport systems that are important for *E. coli* urinary tract colonization, however, it is difficult to understand how central metabolism contributes to pathogen fitness by these approaches because most central pathways are regulated by allosteric mechanisms, *e.g.*, post-translationally. One successful approach has been to construct and utilize central pathway gene deletions to directly assess fitness in the murine model of ascending UTI (18, 43). Using this approach, it has been possible to assess fitness for a number of mutants in each central pathway in *E. coli* (Figure 3). The mutants that have been tested in this way are in, glycolysis (*pgi*, *pfkA*, *tpiA*, *pykA*), pentose phosphate pathway (*gnd*, *talA*, *talB*), Entner Duodoroff (*edd*), TCA cycle (*sdhB*, *fumC*, *frdA*), and gluconeogenesis (*pckA*).

Strains lacking *tpiA* (triose phosphate isomerase) and *pgi* (phosphoglucose isomerase), as well as mutants in irreversible glycolytic steps involving both 6-carbon (*pfkA*; 6-phosphofructokinase transferase) and 3-carbon (*pykA*; pyruvate kinase) demonstrated that neither the preparative or substrate level phosphorylation stages of glycolysis are required during experimental infection (18, 43). Similarly, it was found that a mutant defective in the oxidative branch of the pentose phosphate pathway; phophogluconolactonate (*gnd*), and a mutant defective in gluconate catabolism; 6-phosphoglyconate dehydratase (*edd*), did not demonstrate a fitness defect during experimental UTI (18, 43). *E. coli* encode genes for two transaldolase enzymes, *talA* and *talB*, which function in the non-oxidative pentose phosphate pathway. TalB is the major transaldolase in *E. coli* that transfers a three-carbon moiety from a C_7 molecule to glyceraldehyde-3-P (C_3) to form erythrose-4-P (C_4) and fructose-P (C_6). This stage of the pentose phosphate pathway is reversible, and thus, can be uncoupled from the oxidative decarboxylation reactions that produce NADPH. Interestingly, loss of TalA created a slight fitness advantage and loss of the major transaldolase, TalB, did not affect *E. coli* fitness during UTI, however loss of both TalA and TalB in a double mutant strain created a modest fitness defect (18, 43), suggesting that isomerization of sugars in the pentose phosphate are important during UTI, presumably for nucleoside biosynthesis.

During bacterial growth on gluconeogenic substrates, peptides and certain amino acids that are present in the urinary tract are broken-down into pyruvate, which can be oxidized in the TCA cycle or reduced to fermentative end-products. The resulting oxaloacetate can fuel gluconeogenesis as the substrate for pyruvate carboxykinase (*pckA*) that generates phophoenolpyruvate and bypasses the irreversible glycolytic reaction catalyzed by pyruvate kinase (*pykA*). Mutation of *pckA*, which disrupts gluconeogenesis, results in a significant fitness defect for *E. coli* in both bladder and kidneys (18). The aerobic tricarboxylic acid (TCA) cycle has been proposed to be required for *E. coli* fitness during growth on gluconeogenic substrates present in the urinary tract (18). Specifically, *E. coli sdhB* mutant bacteria have been shown to have fitness defects during UTI (18, 65), suggesting that the reductive TCA cycle may not be operating during host colonization. Mutants lacking fumarate dehydratase (fumarase); *fumC*, and fumarate reductase; *frdA* have also been tested.

It is generally believed that the production of reduced $FADH_2$ during the conversion of succinate to fumarate by succinate dehydrogenase is avoided during fermentation by modification of the TCA cycle to an incomplete reductive pathway where fumarate conversion to succinate by fumarate reductase replaces succinate dehydrogenase activity. The loss of FrdA had no effect on *E. coli* colonization of the urinary tract, while loss of FumC created a fitness defect during bladder colonization (43). Finding that FumC contributes to UPEC fitness during UTI suggests that the TCA cycle may be operating as tool to cope with oxidative stress. It is known that FumC is a fumarase that lacks an iron sulfur cluster and is part of the SoxRS regulon (66). Thus, it is possible that the flexibility of the TCA cycle and related energy pathways as discussed above may reflect a countermeasure against host defense rather than a bona fide indication of aerobic or anaerobic metabolism (13).

Flexible energy pathways

The TCA cycle, in *E. coli* and in nearly all living cells, is an amphibolic pathway because TCA intermediates are important for anabolic processes in addition to catabolism of acetogenic and gluconeogenic carbon sources like amino acids. Both the complete aerobic TCA cycle and the incomplete reductive pathway are intimately linked to energy metabolism and respiration; pyruvate from glycolysis is oxidized to CO_2 concomitant with production of NADH and $FADH_2$, both of which must be re-oxidized because all pathways would cease in that absence of NAD^+. During respiration, the re-oxidation of NADH is coupled to the generation of a proton gradient (ΔpH) that is used, among many processes, to drive ATP production via proton influx through the F_1F_0 ATPase stator. Much like the flexibility of *E. coli* carbon metabolism in the intestine, the modular respiratory chains of *E. coli* can be assembled in a variety of configurations depending on the available terminal electron acceptor and the energy needs of the bacterium (67). In the intestine, the ability to respire aerobically and anaerobically provides a substantial fitness advantage for *E. coli* (68). In the extraintestinal environment, loss of the TCA cycle enzyme succinate dehydrogenase, SdhB, results in a UPEC strain that has reduced fitness *in vivo* (18, 43), suggesting that a complete TCA cycle and aerobic respiration are important in the urinary tract (13). At first approximation, aerobic respiration and acetogenic growth (fermentation) during UTI appear contradictory; however, during rapid growth in the host urinary tract it is likely that acetate metabolism reflects a certain degree of reductive overflow, or an electron sink, to relieve carbon flux through the TCA cycle when the respiratory capacity has been reached (46).

The flexibility of energy metabolism and the modular respiratory chains for *E. coli* are also critical for adapting to respiratory stress (69). Utilization of alternative respiratory chains not only confers flexibility dependent upon the available energy source or terminal electron acceptor (67) but also allows for modulation of the proton motive force (μH^+), the gradient of charge and protons across the cytoplasmic membrane (70). By reducing membrane potential ($\Delta\psi$), *E. coli* can limit or prevent uptake of antibiotics and exist as a bacterial "persister" that can only be eradicated by antibiotics when specific metabolites are present to stimulate respiratory generation of μH^+ (71). This strategy is also utilized by *E. coli* to prevent killing by the bactericidal activities of mammalian phagocytes. In the urinary tract,

extraintestinal *E. coli* infection elicits a massive inflammatory response characterized by neutrophil influx (72, 73) and host production of the antimicrobial peptide LL-37 cathelicidin (74). In response to antimicrobial peptides and acidic pH generated by neutrophils, the PhoPQ regulatory system becomes activated and up-regulates genes that encode electroneutral respiratory chain components, cation import systems, and anion efflux channels to limit respiratory generation of μH^+ and reverse membrane polarity from inside-negative to inside-positive, respectively (75). This control of membrane potential promotes *E. coli* resistance to cationic antimicrobial peptides and acidic pH, which potentiates bacterial survival against bactericidal activities of neutrophils during UTI. Indeed, UPEC lacking PhoP are exquisitely sensitive to polymyxin and acidic pH, and are severely attenuated during acute infection of the bladder (75).

Sensing and responding to environmental cues

Nutritional sensing is important for competing with other bacteria for limiting nutrients in the intestine (23) and could represent a mechanism signaling the arrival into a different host environment such as the urinary tract. Signal transduction by two component regulatory systems, which contain a transmembrane sensor kinase and a cognate response regulator transcription factor, are the best- described mechanism used by bacteria to coordinate gene expression in response to specific external stimuli. In UPEC, disruption of the QseBC two component regulatory system attenuates virulence in *E. coli* within the urinary tract by altering carbon flux through central pathways, which negatively affects the expression of multiple adhesins that are involved in bladder colonization (65). The horizontally acquired *frz* operon has also been shown to link the metabolic capacity of ExPEC with expression of genes required for adherence to the bladder epithelium; the presence of the *frz* operon favors the ON orientation of the invertible type 1 fimbriae promoter (32). UPEC catabolism of D-serine in the urinary tract is also an important signaling mechanism to trigger virulence gene expression (44, 76, 77). These studies suggest that movement of ExPEC from a commensal-like lifestyle in the nutrient- and carbon-rich intestine to the nutritionally poor, nitrogen-rich lower urinary tract would be quickly sensed by changes in metabolism, which would trigger expression of genes required for pathogen colonization of an extraintestinal microenvironment.

The nutrient sensing regulatory system KguSR is a two component system that has been shown to be important for UPEC fitness during UTI (78). This system appears to be involved in the control of utilization of α-ketoglutarate by regulating target genes that encode an α-ketoglutarate dehydrogenase and a succinyl-CoA synthetase (78). The KguSR system was also suggested to be partially controlled by anaerobiosis (78). Interestingly, the canonical anaerobiosis regulator, Fnr, has been shown to play a key role in UPEC fitness during UTI (79). Fnr impacts UPEC fitness by modulating the expression of type 1 and P fimbriae, as well as affecting motility. It is also possible that Fnr is responsible for the apparent effect of anaerobiosis on the KguSR system (79). Another nutritional response that has been observed in UPEC is the regulation of type 1 fimbriae by sialic acid. It has been shown that NanR and NagC inhibit FimB switching from phase off to phase on, resulting in a decrease in the production of type 1 fimbriae (80). Taken together, these findings all

provide examples of nutritional mechanisms whereby UPEC sense the urinary tract environment to modulate expression of virulence factors.

Summary

Future studies aimed at how the presence or acquisition of genomic islands and PAIs affect the metabolome and metabolic capacity of *E. coli* that infect the urinary tract will be important to better define rational drug targets. Investigating changes in carbon flux through central metabolism caused by transcriptional regulators, nutrient-uptake systems, and carbohydrate utilization systems encoded on PAIs will help to identify traits that confer a fitness advantage for UPEC when faced with bacterial competition for nutrients in the human intestine. For *E. coli* transitioning from the carbon-rich intestine to the urinary tract, it is likely that the ratio of carbon:nitrogen, or fluctuations in the intracellular C:N levels plays an important role controlling pathogenicity (Figure 4) (13, 43). Nitrogen metabolism and ammonia generated by catabolism of amino acids can act as a form of long-range interbacterial communication to induce oxidative stress responses and increase resistance to antibiotics (81).

Basic principles of physiology, shared by nearly all living cells, are beginning to be appreciated as playing key roles in processes that are essential for pathogenesis. Bacterial respiration coordinates type three secretion system (TTSS) assembly with movement of *Shigella* from the intestinal lumen to the mucosa (82). *E. coli* modulate their respiratory activity and limit or reverse membrane potential as a protective counter-measure against mammalian host defenses during UTI (75). These studies demonstrate the important link between fundamental bioenergetics and pathogenesis and suggest that energy metabolism might be an important signal used by bacterial pathogens to identify specific host microenvironments. By studying carbon metabolism, key differences between *E. coli* growing in the intestine and colonizing the urinary tract have been identified. Commensal *E. coli* require the Entner-Doudoroff pathway and glycolysis for intestinal colonization; while the pentose phosphate pathway and gluconeogenesis are dispensable (21). In contrast, for *E. coli* infecting the urinary tract, the pathways required for commensal colonization are dispensable while the TCA cycle and gluconeogenesis are required (18). Other aspects of UPEC physiology such as determining if the numerous well-considered or presently uncharacterized fimbriae that predominate in ExPEC strains (83) function as adhesive organelles to promote bacterial adherence in the intestine or in the urinary tract will be beneficial to understand the selective pressures that actively shape and maintain the UPEC pan-genome. Once we better understand how ExPEC are able to transition and adapt to both the intestinal and extraintestinal host microenvironments within the same individual it will be feasible to develop antimicrobials that target pathogenic strains and avoid eradicating beneficial commensal bacteria.

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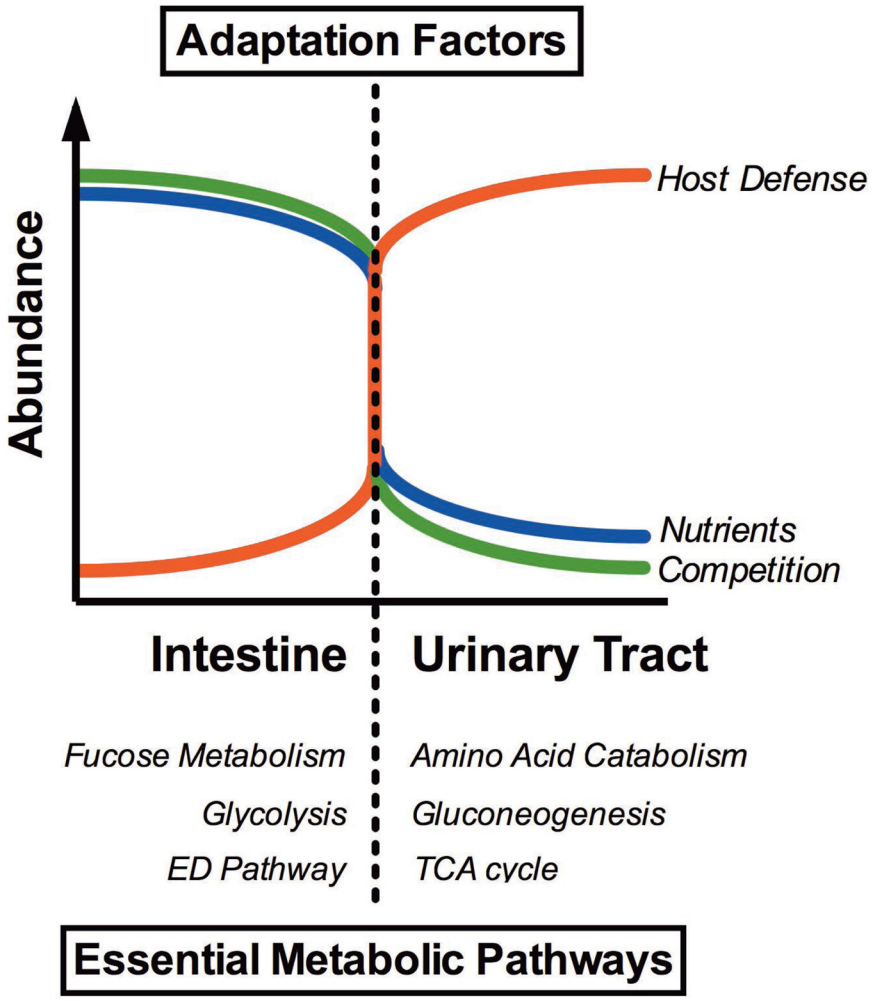

Figure 1. Adaptation of metabolism and basic physiology allows *E. coli* to replicate in diverse host microenvironments

Extraintestinal pathogenic *E. coli* that cause urinary tract infection, bacteremia, sepsis, and meningitis, have adapted to grow as a harmless commensal in the nutrient-replete, carbon-rich human intestine but rapidly transition to pathogenic lifestyle in the nutritionally poor, nitrogen-rich urinary tract. In order to establish a commensal association within the human intestine, adaptive factors such as metabolic flexibility allow *E. coli* to successfully compete for carbon and energy sources with a large and diverse bacterial population. *E. coli* acquires nutrients from the intestinal mucus, including N-acetylglucosamine, sialic acid, glucosamine, gluconate, arabinose, fucose and simple sugars released upon breakdown of complex polysaccharides by anaerobic gut residents. When UPEC transition to the urinary tract, the bacteria encounter a drastic reduction in the abundance of nutrients and bacterial competition. Consequently, to replicate in a new host microenvironment, UPEC utilization of metabolic pathways required for growth in the dilute mixture of amino acids and peptides in the bladder signals the bacterium to elaborate virulence properties to successfully cause invasive disease and survive the onslaught of bactericidal host defenses. These adaptations are a unique and essential characteristic of ExPEC that enable a successful transition

between disparate microenvironments within the same individual. (From Alteri, CJ and HL Mobley. 2012. *Escherichia coli* physiology and metabolism dictates adaptation to diverse host microenvironments. Curr Opin Microbiol. 15:3–9)

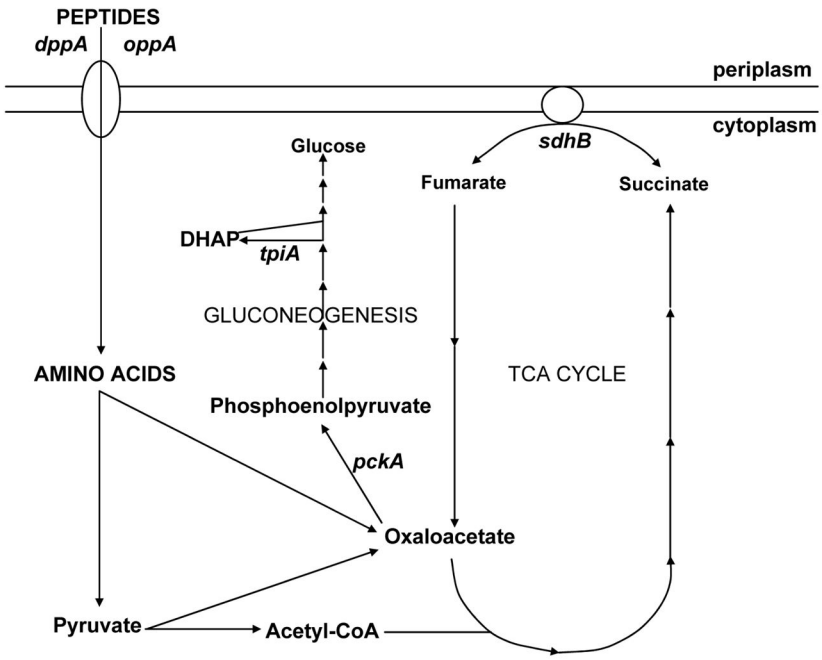

Figure 2. UPEC acquires amino acids and requires gluconeogenesis and the TCA cycle for fitness *in vivo*

Peptide substrate-binding protein genes *dppA* and *oppA* are required to import di- and oligopeptides into the cytoplasm from the periplasm. Short peptides are degraded into amino acids in the cytoplasm and converted into pyruvate and oxaloacetate. Pyruvate is converted into acetyl-CoA and enters the TCA cycle to replenish intermediates and generate oxaloacetate. Oxaloacetate is converted to phosphoenolpyruvate by the *pckA* gene product during gluconeogenesis. Mutations in the indicated genes *dppA*, *oppA*, *pckA*, *sdhB*, and *tpiA* demonstrated fitness defects *in vivo*. (From, Alteri, C., S. Smith, and Harry L.T. Mobley. 2009. Fitness of *Escherichia coli* during Urinary Tract Infection Requires Gluconeogenesis and the TCA Cycle *PLoS Pathogens*. May 5:e1000448)

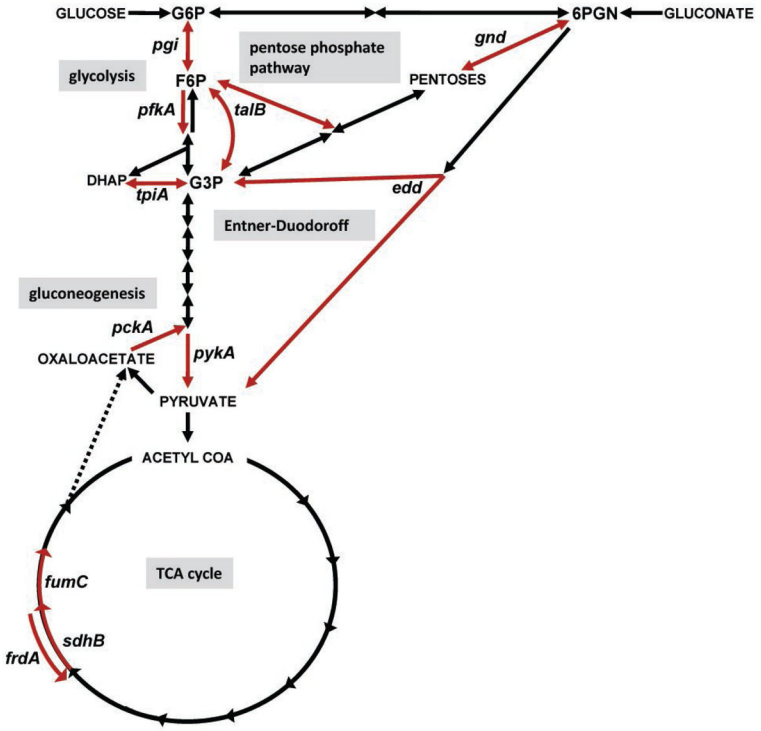

Figure 3. Diagram of central metabolism and map of the specific pathways disrupted by targeted mutations in uropathogenic *E. coli*

Carbon sources or biochemical intermediates shared between pathways are indicated in capital letters or abbreviated: G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; G3P, glyceraldehyde-3-phosphate; 6PGN, 6-phosphogluconate. Reactions are denoted with arrows. Specific reactions (red arrows) were targeted by deletion or insertion in *E. coli* CFT073. In glycolysis: *pgi*, glucose-6-phosphate isomerase; *pfkA*, 6-phosphofructokinase transferase; *tpiA*, triosephosphate isomerase; *pykA*, pyruvate kinase; in pentose phosphate pathway: gnd, 6-phosphogluconate dehydrogenase; *talB*, transaldolase; in Entner-Duodoroff pathway: *edd*, 6-phosphogluconate dehydratase; in gluconeogenesis: *pckA*, phosphoenolpyruvate carboxykinase; and in the TCA cycle: *sdhB*, succinate dehydrogenase; *fumC*, fumarate hydratase; *frdA*, fumarate reductase. (From, Alteri, Christopher J., Stephanie Himpsl, and Harry L.T. Mobley. 2015. Preferential Use of Central Metabolism in vivo Reveals a Nutritional Basis for Polymicrobial Infection. PLoS Pathogens 11:e1004601.)

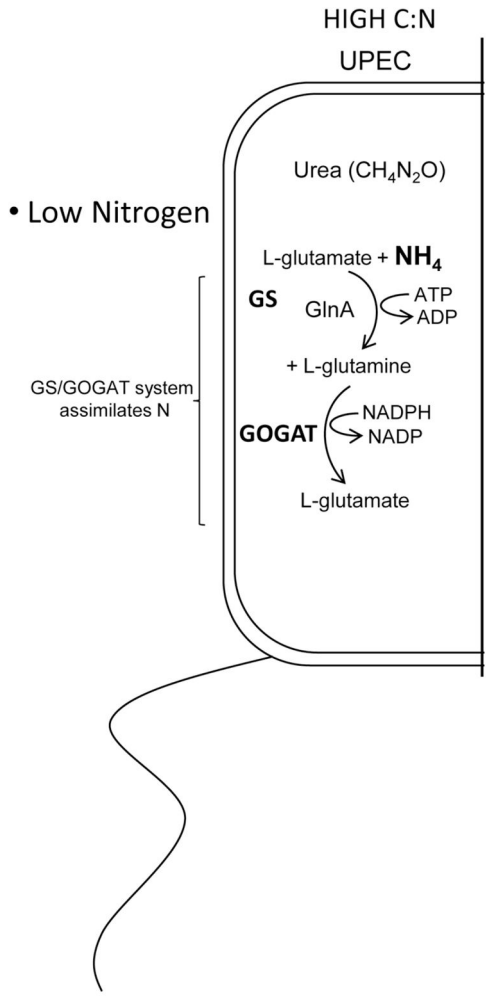

Figure 4. Model describing the C/N ratio within the urinary tract for *E. coli*

The urinary tract environment has a low C/N ratio due to the dilute mixture of amino acids and peptides as the primary carbon source and the abundance of urea in urine providing a substantial nitrogen contribution. *E. coli* is unable to utilize or sense the nitrogen sequestered in urea because it lacks urease, which liberates ammonia from urea. This results in *E. coli* activation of the glutamine synthetase and glutamate oxo-glutarate aminotransferase system (GS/GOGAT) to assimilate nitrogen. (From, Alteri, Christoper J., Stephanie Himpsl, and Harry L.T. Mobley. 2015. Preferential Use of Central Metabolism in vivo Reveals a Nutritional Basis for Polymicrobial Infection. PLoS Pathogens 11:e1004601)