REVIEW / SYNTHÈSE

Acid stress response in enteropathogenic gammaproteobacteria: an aptitude for survival[1]

Boyu Zhao and Walid A. Houry

Abstract: Enteric bacteria such as *Escherichia coli* have acquired a wide array of acid stress response systems to counteract the extreme acidity encountered when invading the host's digestive or urinary tracts. These acid stress response systems are both enzyme and chaperone based. The 3 main enzyme-based acid resistance pathways are glutamate-, arginine-, and lysine-decarboxylase pathways. They are under a complex regulatory network allowing the bacteria to fine tune its response to the external environment. HdeA and HdeB are the main chaperones involved in acid stress response. The decarboxylase systems are also found in *Vibrio cholera*, *Vibrio vulnifus*, *Shigella flexneri*, and *Salmonella typhimurium*, although some differences exist in their functional mechanism and regulation.

Key words: acid stress, glutamate decarboxylase, arginine decarboxylase, lysine decarboxylase, HdeA/HdeB.

Résumé : Les bactéries entériques comme *Escherichia coli* ont acquis une vaste gamme de systèmes de réponse au stress acide afin de contrecarrer l'acidité extrême rencontrée lorsqu'elles envahissent les systèmes digestifs ou urinaires de leurs hôtes. Ces systèmes de réponse au stress acide reposent sur des enzymes ou des chaperons. Les trois principales voies de résistance à l'acidité basées sur des enzymes sont les voies des glutamate-, arginine- et lysine décarboxylases. Elles sont placées sous le contrôle d'un réseau régulateur complexe qui permet à la bactérie de régler finement sa réponse à l'environnement externe. HdeA et HdeB sont les deux chaperons principaux impliqués dans la réponse au stress acide. Les systèmes des décarboxylases sont aussi trouvés chez *Vibrio cholera, Vibrio vulnifus, Shigella flexneri* et *Salmonella typhimurium,* même s'il existe quelques différences entre leur mécanisme de fonctionnement et leur régulation.

Mots-clés : stress acide, glutamate décarboxylase, arginine décarboxylase, lysine décarboxylase, HdeA/HdeB.

[Traduit par la Rédaction]

Introduction

Seemingly small and vulnerable, bacteria possess an extraordinarily complicated set of stress response mechanisms that give them the ability and resilience to survive, or even thrive, in harsh conditions. Acid resistance in enteric bacteria such as *Escherichia coli* is one important example of such an adaptation. These bacteria can colonize the intestines of their host organism, including humans, and cause infection. In the process, they inevitably have to pass through the gastric acid in the stomach (pH 2.5), which serves as a natural antibiotic barrier. However, despite being neutrophiles, they can survive in this hostile acidic condition. Therefore, understanding the complex regulatory mechanisms and pathways of the bacterial acid stress response is crucial to developing strategies for controlling bacterial infection. In this review, we will discuss the acid resistance systems in enteric bacteria, focusing primarily on describing the mechanism of function of the enzymes and chaperones involved in the acid resistance pathways. At first, we will describe the decarboxylase-based and chaperone-based pathways in *Escherichia coli*, as they are the most extensively studied. Subsequently, we will compare and contrast the acid stress systems among 5 enteric bacterial species: *Escherichia coli*, *Vibrio cholera*, *Vibrio vulnifus*, *Shigella flexneri*, and *Salmonella typhimurium*.

Enzyme-based acid stress response systems in *E. coli*

Five acid resistance (AR) pathways, AR1–AR5, are known in *E. coli*. (Kashiwagi et al. 1992; Foster 2004) (Fig. 1). The AR1 pathway, though poorly understood, is activated when cells are placed in minimal media at pH 2.5

Received 17 September 2009. Revision received 20 November 2009. Accepted 7 December 2009. Published on the NRC Research Press Web site at bcb.nrc.ca on 18 March 2010.

B. Zhao and W.A. Houry.[2] Department of Biochemistry, University of Toronto, Toronto, ON M5S 1A8, Canada.

[1]This paper is one of a selection of papers published in this special issue entitled "Canadian Society of Biochemistry, Molecular & Cellular Biology 52nd Annual Meeting — Protein Folding: Principles and Diseases" and has undergone the Journal's usual peer review process.

[2]Corresponding author (e-mail: walid.houry@utoronto.ca).

without an external supply of any amino acid after the cells are grown at pH 5.5 to stationary phase in buffered Luria broth (Foster 2004). This pathway requires the alternative signal factor σ^S and cAMP receptor protein (CRP) to function. Because CRP is involved, the system is repressed by glucose (Foster 2004). The AR2–AR5 pathways are all amino acid decarboxylase-based pathways. In general, they consist of a decarboxylase enzyme that is induced by low pH and the presence of a specific amino acid, as well as an antiporter. The enzymes involved in AR2, AR3, and AR4 are the glutamate, arginine, and lysine decarboxylases, respectively. The AR2 and AR3 pathways enable bacteria to survive in extreme acidic environments (e.g., pH 2.5) (Foster 2004). The AR4 and AR5 pathways allow *E. coli* cells to survive in moderately acidic environments (e.g., pH 4.5). AR5 consists of the inducible ornithine decarboxylase SpeF, which has a pH activity optimum of 6.9, and the ornithine–putrescine antiporter PotE (Kashiwagi et al. 1992; Foster 2004). AR5 is not as well studied as AR2–AR4 and will not be discussed.

AR2: the glutamate decarboxylase system

This system has been extensively studied and constitutes the major acid response system in *E. coli* under extreme acidic conditions. The system consists of paralogous GadA and GadB decarboxylases and an inner-membrane antiporter GadC. GadA and GadB are pyridoxal 5′-phosphate (PLP)-dependent enzymes that convert glutamate to gamma-amino butyric acid (GABA) and carbon dioxide (CO_2) in a reaction that consumes a cytoplasmic proton (Bearson et al. 1997; Foster 2004) (Fig. 1). GABA is transported out of the cell by the inner membrane antiporter GadC in exchange for more glutamate (Bearson et al. 1997).

Structures of the glutamate decarboxylases

The structures of both GadA and GadB have been solved by X-ray crystallography (Capitani et al. 2003; Dutyshev et al. 2005). The two isozymes differ in their primary sequences at only 5 residues and are therefore very similar in structure. They form 330 kDa hexamers assembled from trimerization of GadA(B) dimers. The GadA(B) monomer can be divided into 3 domains: the N-terminal domain, the large domain (PLP binding domain), and the C-terminal small domain (Fig. 2). The N-terminal domain is critical for the function of GadB because it is responsible for the preferential association of GadB with the inner membrane when pH is lowered. Deletion of the first 14 residues of GadB diminishes its ability to migrate to the inner membrane. By comparing the structures of GadB at neutral (pH 7.6) and acidic pHs (pH 4.6), the N-terminal domain is found to undergo a conformational change from a disordered state containing little secondary structure at neutral pH to an α-helix at acidic pH (Capitani et al. 2003) (Fig. 3). This α-helix is oriented perpendicularly to the subunit surface. As a result, the active (low pH) form of GadB hexamer has a 3-helical bundle on each of its 2 opposing surfaces (Fig. 3). The bundles have a hydrophobic core and are charged on the outside. There are 3 Asp residues and 1 Glu residue in the first 15 residues of GadB. At least two of them are protonated upon acidification, resulting in the conformational changes in GadB N-terminus (Capitani et al. 2003).

More interestingly, halide ions such as Cl^- are found, by X-ray crystallography, to be able to bind to the bottom of the C-terminus of each of the N-terminal helices in the 2 triple-helix bundles (Gut et al. 2006). The binding fixes the turn formed by residues 16–19 and, as a result, stabilizes the triple α-helix bundle required for GadB hexamer interaction with the inner membrane (Gut et al. 2006). This finding is significant because it shows an additional function of Cl^- ion on top of its use to protect the membrane potential during extreme acid stress (see discussion on the role of Cl^- in acid stress below).

The X-ray structure of GadA hexamer was only solved at pH 4.6 and the N-terminal helix structure was resolved at that pH. There is no structure of GadA at a higher pH. Therefore, although similar biochemical experiments to GadB are not yet performed with GadA, it is highly likely that N-terminal domain in GadA also undergoes a conformational change from a disordered state at neutral pH to an α-helix at acidic pH.

The active site of GadB (as well as of GadA) is in the PLP-binding large domain. Lys276, in GadB (GadA), forms a Schiff base linkage with the C4 atom of the pyridine ring of PLP. Unlike other PLP-dependent enzymes, GadB uses Gln163 instead of an aromatic residue for a stacking interaction with the pyridine ring of PLP (Capitani et al. 2003). When the glutamate substrate interacts with the active site of GadB, it is held in place by hydrogen binding of its γ-carboxylate group with the protein Phe63 main chain, the Thr62 side chain, and the carboxylate side chain of Asp86 of the neighboring subunit. This binding explains the maximum activity of GadB at low pH, since either the Glu substrate or Asp86 of GadB must be protonated for this interaction to occur. In addition, an Arg422 residue that binds the α-carboxylate of the substrate in many PLP-dependent enzymes is kept away from interacting with the Glu substrate in GadB, so that it does not interfere with the decarboxylation process (Capitani et al. 2003).

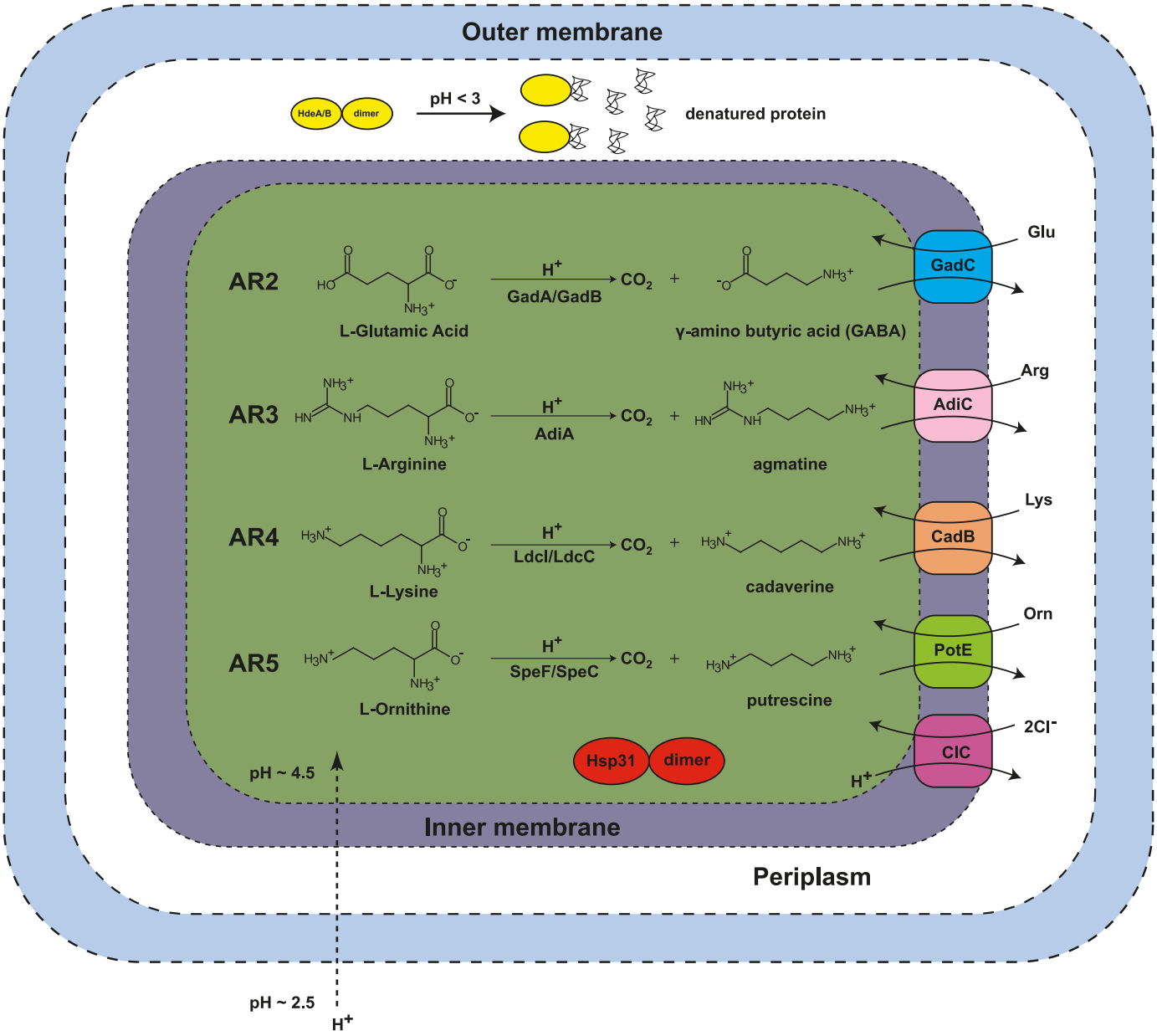
When the pH increases back to neutral, GadB undergoes a stepwise conformational change to its inactive form. At first, each of the N-terminal triple helical bundles unfold independently. When both are unfolded, an aldimine structure (imine derived from an aldehyde) forms between the imadizole ring of His465 at the C-terminal end of GadA/B and Lys276-PLP imine to close the active site (Gut et al. 2006). This covalent adduct is the 340 nm-absorbing chromophore that is the signature of the inactive form of GadB (Gut et al. 2006). Hence, at neutral pH (pH 7.6), each active site funnel is blocked by the C-terminus of the same subunit and by a β-hairpin from the neighboring subunit.

Regulation of the glutamate decarboxylase system

As the most effective acid stress response pathway under extreme acid stress conditions, the AR2 system is intricately regulated. To date, there are over 20 proteins and 3 small non-coding RNAs that are identified as regulating the Gad system (Fig. 4A). The proteins and factors include CRP, Dps, EvgA/S, GadE, GadX, GadW, H-NS, Lon, PhoP/Q, RNaseE, σ^{70}, σ^s, SspA, TrmE, TopA, TorS/R, and YdeO. The 3 small non-coding RNAs are DsrA, GadY, and GcvB.

In the *E. coli* genome, the *gadA* and *gadB* genes are located 2100 kb apart (Fig. 4A). The *gadC* gene is located

Fig. 1. Schematic diagram of the acid resistance (AR) systems in *E. coli*. The amino acid and decarboxylation products are shown in chemical notation and the proteins responsible for the reactions are shown under the reaction arrows. All the decarboxylation reactions consume a proton and release carbon dioxide. AR1 is not shown because the system is not well characterized. The HdeA/B and Hsp31 chaperones are represented by ovals. This figure is adapted from Gajiwala and Burley (2000), Zhao et al. (2003), and Foster (2004).

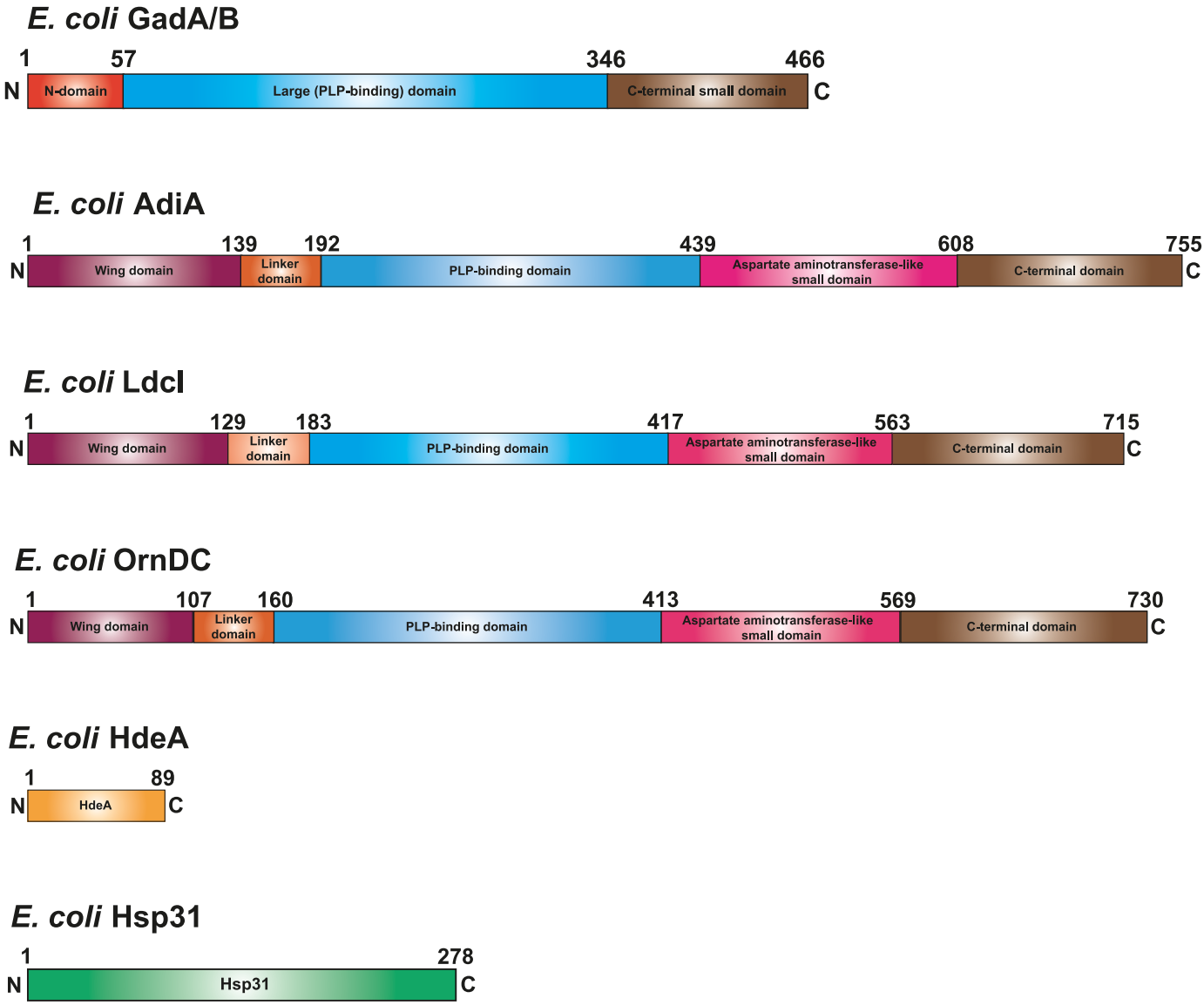


downstream of *gadB* and they form an operon (Dutyshev et al. 2005). The central transcriptional activator of the *gadA* and *gadBC* genes is GadE (Ma et al. 2003; Foster 2004). GadE induces the expression of the Gad system by binding to a 20 bp sequence, termed the *gad* box, which is located around 60 bp upstream of the transcription start sites of *gadA* and *gadBC* (Castanie-Cornet and Foster 2001; Ma et al. 2002).

Apart from the Gad system, GadE also serves as a global transcriptional activator for many genes. When overexpressed, GadE is found to induce genes involved in stress response (e.g., *somC*, *hdeA*, and *ycgG*), in biosynthesis of glutamate (e.g., *gltD* and *gltH*), and also membrane components (e.g., *rcsA* and *rfaG*) (Hommais et al. 2004).

Other protein factors regulate, directly or indirectly, the expression of GadE depending on the growth phase of the cells and on the media (Fig. 4A). Recently, it was found that there is a 750 bp regulatory region upstream of the transcription start site of the *gadE* gene (Sayed and Foster 2009). This region contains 3 promoters (P1, P2, and P3) that allow different regulators to bind and produce 3 *gadE* transcripts: T1, T2, and T3. T1 starts at –124, T2 at –324, and T3 at –566 bases from the *gadE* start codon. The P1, P2, and P3 promoters are located about 200 bp upstream of the start of each transcript. P1 is where GadE acts to auto induce itself in minimal medium containing glucose (Ma et al. 2004; Sayed and Foster 2009). This auto activation also requires σ^s, the alternative sigma factor responsible for the

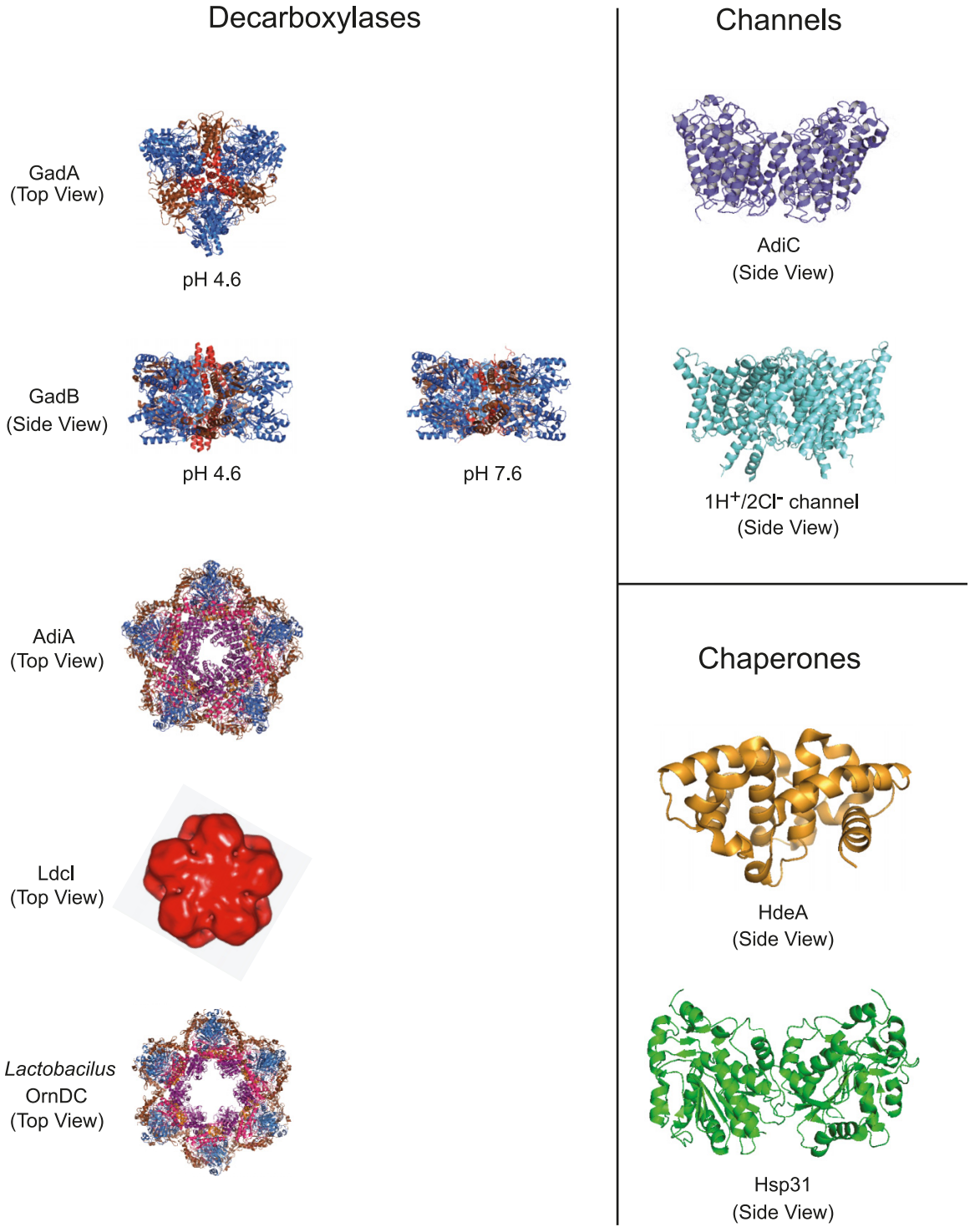
Fig. 2. Domain arrangement of the acid stress induced amino acid decarboxylases and chaperones. The domain boundaries for *E. coli* GadA/B, AdiA, HdeA, and Hsp31 are based on the solved X-ray structures. Since the structures of *E. coli* LdcI and ornithine decarboxylase (OrnDC) are not yet solved, their domain boundaries are defined based on sequence alignment with *Lactobacillus* 30a OrnDC whose X-ray structure has been solved (Momany et al. 1995).



transcription of many stress response genes in stationary phase, and another unidentified factor. P2 and P3 are activated by GadX (YhiX) and GadW (YhiW) during stationary phase growth and by the EvgA/S and YdeO pathway during exponential phase growth in minimal medium at acidic pH (Ma et al. 2004).

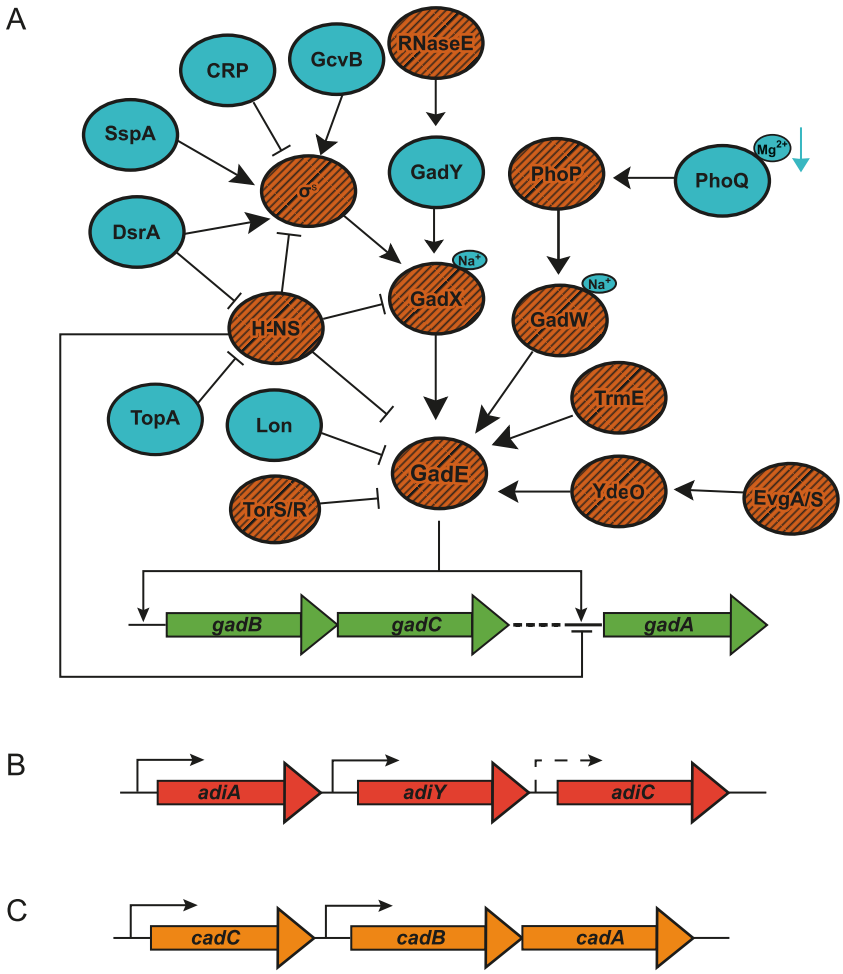
GadX and GadW are both AraC-like regulators; AraC is a well-studied transcription activator of the arabinose operon that encodes proteins involved in metabolizing L-arabinose. Along with σ^s, GadX and GadW are important for inducing the Gad system in stationary phase cells grown in either minimal or rich media (Ma et al. 2002). The transcription of *gadX* is induced by σ^s (Fig. 4A). CRP and H-NS proteins are two repressors of *gadX* transcription. CRP represses the production of σ^s during normal cell growth and, thus, indirectly represses *gadX* transcription (Castanie-Cornet and Foster 2001). H-NS is a major component of bacterial chromatin. It preferentially binds AT-rich DNA sequences, often found in *E. coli* promoters, to repress expression of downstream genes. H-NS is a repressor of *gadA*, *gadE*, and *gadX*, but not of *gadBC* (Giangrossi et al. 2005). Several in vitro experiments have shown that GadW and GadX can bind directly to the promoter regions of *gadA* and *gadBC* and induce their expression through different mechanisms (Ma et al. 2002; Giangrossi et al. 2005; Tramonti et al. 2006). However, it seems that in vivo GadX and GadW activate the Gad system indirectly by activating *gadE* transcription (Fig. 4A), because overexpression of GadX cannot induce *gadA* or *gadBC* in a *gadE* mutant background (Ma et al. 2003; Gong et al. 2004; Ma et al. 2004; Sayed et al. 2007). Moreover, overexpression of GadE seems to diminish the requirement for GadX and GadW in *E. coli* acid resistance, but not vice versa (Ma et al. 2003). Therefore, the in vitro results do not exactly agree with the in vivo results. So

Fig. 3. X-ray structures of the acid stress induced amino acid decarboxylases, antiporters, and chaperones. The domains of the decarboxylases and chaperones are colour-coded according to Fig. 2. The structures of *E. coli* GadA (PDB ID:1XEY (Dutyshev et al. 2005)), *E. coli* GadB (1PMO, inactive form at pH 7.6; 1PMM, active form at pH 4.6 (Capitani et al. 2003)), *E. coli* AdiA (2VYC (Andréll et al. 2009)), *E. coli* LdcI (EM image (Snider et al. 2006)), and *Lactobacillus* 30a OrnDC (1ORD (Momany et al. 1995)) are displayed in the same column for comparison. The X-ray structures of the *E. coli* AdiC antiporter [3H5M (Gao et al. 2009)] and *E. coli* ClC Cl^- channel (1KPK (Dutzler et al. 2002)) are also shown. *Escherichia coli* HdeA (1DJ8 (Gajiwala and Burley 2000)) and Hsp31 (1ONS (Zhao et al. 2003)) are shown in their physiological dimeric form. (Note: the full-colour versions of Figures 2 and 3 are available from http://bcb.nrc.ca.)



far, the exact mechanism of the GadX/W/E-mediated activation has not been elucidated. It is probable that subsequent to binding of GadX/W to the *gadA/BC* promoters, cooperation with GadE is still required for full activation of the Gad system (Sayed et al. 2007). Moreover, the mechanism of GadX/W/E activation maybe different depending on the en-

Fig. 4. Schematic depiction of the gene organization and regulation of the different decarboxylase systems. (A) Regulation of the glutamate decarboxylase system. Arrows indicate activation, whereas T-shaped lines indicate repression. Cross-hatching indicates that the proteins affect the gene transcription of the connected protein; lack of cross-hatching indicates other mechanisms of regulation such as through protein–protein, protein–DNA, or RNA–RNA interaction. Further details are given in the text. (B) Gene organization of the arginine decarboxylase system. (C) Gene organization of the lysine decarboxylase system.



vironmental conditions under which the Gad system is induced (Sayed et al. 2007).

EvgA/S and YdeO specifically enhance the transcription of *gadE* during exponential growth in minimal medium with glucose (Fig. 4A). EvgS is a membrane-bound sensor kinase, whereas EvgA is the response regulator in the 2 component system and YdeO is an AraC-like transcription regulator. EvgA and YdeO have additive effects on the activation of *gadE* transcription. EvgA can also indirectly activate *gadE* by activating YdeO through phosphorylation (Ma et al. 2004). Another two component system that regulates the transcription of *gadE* is TorS/R (Fig. 4A). They are found to repress *gadE* transcription (Bordi et al. 2003). TorS/R induce genes involved in using alternative electron donor in the absence of oxygen.

Lon protease constitutively degrades the GadE protein, even under acid induction (Heuveling et al. 2008). Lon acts as a major cellular protein quality control in *E. coli*. Its effect on GadE can rapidly terminate the acid stress response when pH goes back to neutral, and it also prevents overexpression of acid resistance genes in stationary phase cells (Heuveling et al. 2008). Timely termination of the Gad system is important for restoring the intracellular pH with the help of the ClC proton-chloride $1H^+/2Cl^-$ antiporter (see discussion below).

TrmE (MnmE) is a GTPase involved in tRNA modification. Its GTPase domain is critical for regulating *gadE* transcription and also the transcription and translation of *gadA* and *gadBC* in stationary phase cells grown in rich medium with glucose (Gong et al. 2004). σ^{70} is thought to initiate *gadA* and *gadBC* expression in minimal media in an *hns*Δ *rpoS*Δ mutant (Waterman and Small 2003*b*).

SspA, TopA, and Dps are also involved in the regulation of the Gad system (Fig. 4A). SspA, or stringent starvation protein A, can reduce the H-NS levels post-transcriptionally in stationary phase cells. Because H-NS inhibits the transcription of *rpoS*, SspA is required to activate the transcription of *rpoS* and, hence, genes that are further regulated downstream such as *gadX* (Hansen et al. 2005). Topoisomerase I (TopA) mutant strain has reduced levels of GadA/B and is acid sensitive. Because TopA relieves hypernegative supercoiling in transcription elongation, it is thought that TopA is required to counteract H-NS repression of *gadA/B* genes (Stewart et al. 2005). Dps stands for DNA-binding

protein in starved cells. It keeps the integrity of the bacterial chromosome under low pH by binding to DNA directly, thereby participating in acid stress protection (Jeong et al. 2008).

The expression of the Gad system is also influenced by the concentration of metal ions, such as Mg^{2+} and Na^{+} (Fig. 4A). A decrease in external Mg^{2+} levels is sensed by the Mg^{2+} membrane sensor PhoQ of the PhoP/Q two-component system. PhoQ then phosphorylates and activates PhoP, which promotes transcription of *gadW*. Deletion of *phoP* results in an acid sensitive *E. coli* strain (Zwir et al. 2005). Na^{+} ions modulate the activity of GadX and GadW post-transcriptionally. GadX activates the transcription of *gadE* only when the concentration of Na^{+} ions is high (Richard and Foster 2007).

Finally, there are 3 small non-coding RNAs that regulate the Gad system: DsrA, GadY, and GcvB (Fig. 4A). DsrA stabilizes the *rpoS* mRNA to enhance its translation and increases *hns* mRNA turnover to block translation of H-NS. It does so by sequence-specific RNA–RNA interactions. Deletion of *dsrA* compromises *E. coli* acid resistance ability (Lease et al. 2004). The GadY small RNA is encoded in an intergenic region between *gadX* and *gadW* (Fig. 5), and its transcription is dependent on σ^{s}. GadY transcripts exist in 3 different forms. GadY indirectly induces *gadE* expression by base-pairing with the 3′UTR of the *gadX* mRNA to increase its stability and translation (Opdyke et al. 2004). The expression of GadY is reduced when the *rne* gene, which codes for RNaseE, is disrupted. RNaseE is thought to be involved in the processing of a putative unstable GadY precursor transcript into the mature forms of the GadY transcripts. This mutant also has decreased levels of *gadA* and *gadB* expression and lower survival rate at pH 2.5. Thus, this essential endoribonuclease is also needed for the proper function of the Gad system (Takada et al. 2007). The third small RNA, GcvB, positively regulates acid resistance by increasing the expression level of *rpoS*. The mechanism of how GcvB does so is unknown, as GcvB does not directly affect *rpoS* transcription, nor does it interact with H-NS, GadW, or CRP (Jin et al. 2009).

Based on the previous discussion, it can be seen that the Gad system (AR2) is under the control of an extraordinarily complex regulatory network that integrates multiple environmental cues and physiological adaptations (Fig. 4A).

AR3: the arginine decarboxylase system

Like AR2, this system also enables *E. coli* to survive in extreme acid environments (e.g., pH 2.5). The AR3 pathway consists of the arginine decarboxylase AdiA and an antiporter, AdiC (Foster 2004). By a similar mechanism to AR2, the AR3 pathway utilizes arginine to increase intracellular pH (Fig. 1). AdiA converts arginine into agmatine (Agm) and CO_2. The agmatine is then transported out of the cell by AdiC in exchange for more arginine (Iyer et al. 2003). Both AR2 and AR3 pathways are able to increase the intracellular pH of the bacteria to approximately 4.5 compared to a pH of 3.5 in the absence of these genes under extreme acid stress when the external pH is 2.5 (Foster 2004).

The structures of both AdiA and AdiC have been solved by X-ray crystallography (Andréll et al. 2009; Gao et al. 2009) (Fig. 3). The AdiA monomer has five domains, from N- to C-terminus: the wing domain, the linker domain, the PLP-binding domain, the aspartate aminotransferase (AspAT)-like small domain, and the C-terminal domain (Andréll et al. 2009) (Fig. 2). Above pH 6.5, AdiA exists as an inactive dimer. When pH drops below pH 6.0, five dimers assemble to become an 800 kDa active AdiA decamer. Interaction between the N-terminal wing domains in neighboring AdiA dimers is responsible for this association. The wing domain contains many acidic residues exposed at the dimer surface. At neutral pH, the wing domains carry a net negative charge and repulse each other, thus, preventing dimer association. At acidic pH, the surface charges are partially neutralized to facilitate the formation of decamers. Thus, the AdiA decamer is not only the active form of AdiA, but also serves as a proton buffer as pH decreases (Andréll et al. 2009).

There are two active sites per AdiA dimer at the dimer interface buried from the dimer surface. The cofactor PLP sits at a cleft formed by the C-terminal domain of one monomer and the PLP-binding domain of the other monomer. PLP is stabilized by multiple interactions in the PLP-binding domain: the PLP phosphate group is stabilized by the dipole of the N-terminus of an α-helix. Lys386 covalently binds to the C4 atom of the PLP pyridine ring which is stacked between the side chains of His255 and Ala349 (Andréll et al. 2009; Gao et al. 2009). When the substrate (arginine) is not bound, the active site is open. PLP is visible from a cleft formed at the active site. The ridge of the cleft at the C-terminal domain is lined with negatively charged residues, including three Glu residues that serve to interact with the positively charged substrate (Andréll et al. 2009). The active site, upon binding of substrate, then closes to bring a Glu from one monomer close to an Asp residue from the other monomer. These two residues then coordinate the guanidinium group of the arginine substrate. The AdiA structure thus explains the maximal activity of AdiA at pH 5.2, since this pH ensures that both the Glu and Asp residues retain their negative charge for substrate coordination, and the acidic surface of the AdiA dimer is optimally neutralized to allow assembly of the AdiA decamers (Andréll et al. 2009).

The X-ray structure of the arginine/agmatine antiporter AdiC from wildtype and pathogenic O157:H7 *E. coli* strains was recently solved (Fang et al. 2009; Gao et al. 2009) (Fig. 3). AdiC exists as a homodimer in the cytoplasmic membrane and each monomer has 12 transmembrane segments (TM). The AdiC structures in these two *E. coli* strains are otherwise identical except for a position shift of 3–4 amino acids in TM 6–8 between the two structures (Fang et al. 2009). The homodimeric interface is formed mainly by interactions between hydrophobic amino acids from TM11 of one monomer and nonpolar residues from TM12 of the other. At the default state, AdiC opens to the periplasm, exposing its central cavity where the active site lies (Fig. 3). The active site contains many conserved residues among antiporters in the four decarboxylase pathways. It is lined with Tyr, Asn, Ser, and Glu residues from multiple TMs that bind positively charged Arg or Agm. Binding of Arg and Agm generally involves different tyrosine residues, but Y93 is required for both.

The proposed transport mechanism by AdiC is as follows:

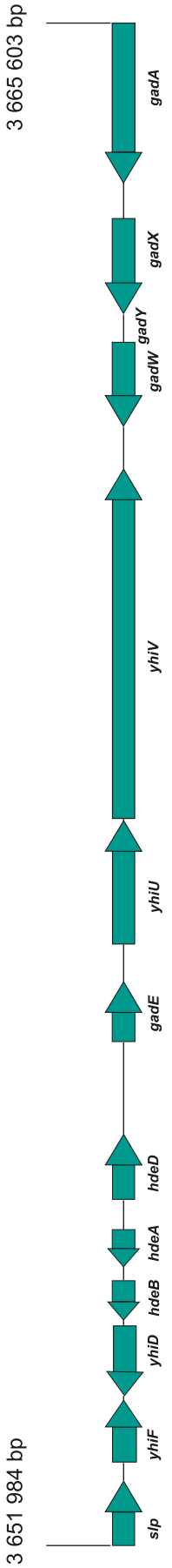


Fig. 5. The acid fitness island in *E. coli*. The island is located at 3651984–3665603 bp on the *E. coli* genome. The *yhiUV* genes are also termed *mdtEF*. The figure is drawn to scale.

after Arg from the extracellular milieu binds at the central cavity of AdiC, the Arg is occluded from the periplasm. AdiC then undergoes a conformational change to become open to the cytoplasm and Arg is displaced by Agm. AdiC then closes over to occlude Agm from the cytoplasm until AdiC switches back to the default conformation to release Agm to the periplasm. A number of conserved polar amino acids located along the central axis are thought to constitute the route of substrate transport. The Glu208 is proposed to be the pH sensor. With a pK_a of 4.25, Glu208 is predominantly protonated at low pH, such as in the stomach (pH 2). At this pH, it can bind the head group of its Arg substrate, which would have no net charge at pH 2 with the positively charged α-amino group offsetting the negatively charged α-carboxyl group. Once facing the cytoplasm (pH 4–5), Glu208 is deprotonated and can bind the positively charged head group of Agm. The TM6 and TM10 of AdiC are proposed to serve as the primary switch between different AdiC conformations during substrate transport (Gao et al. 2009).

The regulation of the AR3 pathway is not as extensively studied as AR2. So far, only a CysB protein is shown to act as an activator of the *adiA/C* genes in anaerobic conditions in complex medium at low pH (Shi and Bennett 1994; Foster 2004). The AraC-like regulator AdiY, located between *adiA* and *adiC* in the genome (Fig. 4B), can also enhance the expression of the two genes when overexpressed. However, AdiY is not essential for the function of the Adi system in *E. coli*, in that the transcription of the *adiA/C* genes is not affected by mutations in AdiY (Foster 2004).

AR4: the lysine decarboxylase system

In contrast to AR2 and AR3, AR4 functions in moderate acidic conditions (pH 5). The AR4 system consists of the lysine decarboxylase LdcI (CadA) and a lysine–cadaverine antiporter, CadB (Soksawatmaekhin et al. 2004). The two genes are organized into a *cadBA* operon (Watson et al. 1992) (Fig. 4C). LdcI has a pH optimum of 5.7 and converts lysine into cadaverine and CO_2 (Soksawatmaekhin et al. 2004) (Fig. 1).

The domain organization of LdcI is the same as that of AdiA (Momany et al. 1995). In its active form, LdcI forms a decamer composed of 5 dimers. LdcI has recently been visualized by electron microscopy (EM) (Snider et al. 2006) (Fig. 3). The LdcI decamer is shown to have a 5-fold symmetry and to be composed of 2 stacked rings with a skew of about 35° with respect to one another. LdcI interacts tightly with RavA, a AAA+ (ATPase associated with various cellular activities) MoxR-family chaperone-like adenosine triphosphatase (ATPase) (Snider et al. 2006), which is induced in stationary phase cells by σ^S. LdcI and RavA form a cage-like structure composed of 5 RavA hexamers bridging 2 LdcI decamers. Interaction of RavA with LdcI seems to enhance RavA ATPase activity but has no effect on the activity of LdcI (Snider et al. 2006).

Although the structure of CadB is not solved, site-specific mutagenesis studies have identified critical residues that are

involved in the uptake or excretion of cadaverine, which include Glu (76, 204, 377, and 408), Asp (185 and 303), Tyr (57, 73, 89, 90, 107, 174, 235, 246, 310, 366, 368, and 423), and Trp (41 and 43). Cys370 is important for both uptake and excretion of cadaverine (Soksawatmaekhin et al. 2006).

Upstream of the *cadBA* operon is the *cadC* gene that encodes the transcription activator of the operon (Fig. 4C). CadC is a 58 kDa protein that binds –144 to –112 and –89 to –59 bp from the transcription start site of the *cadBA* promoter to activate the operon. CadC is constitutively expressed in *E. coli* (Neely and Olson 1996). The protein can be divided into 3 domains: the N-terminal DNA-binding domain, the transmembrane domain, and the C-terminal periplasmic domain (Neely et al. 1994). The C-terminal periplasmic domain is responsible for sensing pH changes, although the mechanism for this is not yet known (Dell et al. 1994). The presence of lysine is sensed directly by LysP, a lysine permease that has 12 transmembrane segments. In the absence of lysine, LysP inhibits CadC via an interaction between the transmembrane segments of LysP and a cluster of 6 aromatic amino acids, in particular F165, in the transmembrane domain of CadC (Tetsch et al. 2008). In addition, Arg265 is the only residue in the periplasmic domain that is involved in the lysine-dependent activity of CadC (Neely et al. 1994). When lysine is abundantly available, LysP activity is repressed and CadC is released to activate the transcription of *cadBA* (Neely and Olson 1996; Tetsch et al. 2008).

The *cadBA* operon is induced by low pH, lysine, and anaerobiosis (Neely and Olson 1996). Kinetic studies showed that induction of *cadBA* operon is slower when cells are exposed to lysine and low pH simultaneously than when they are previously adapted to either (Neely and Olson 1996). H-NS is a repressor of the *cadBA* operon under normal conditions, possibly by affecting DNA topology (Dell et al. 1994). The expression of *cadBA* is also repressed by the accumulation of cadaverine, which binds to the C-terminal periplasmic domain of CadC (Neely et al. 1994; Tetsch et al. 2008).

The role of chloride and potassium ions in acid resistance

One issue *E. coli* needs to deal with upon encountering acidic conditions is the regulation of transmembrane potential, which is disrupted in extreme acid (e.g., at pH 2) (Foster 2004). The strategy that *E. coli* has developed is to reverse the electrical membrane potential from approximately –90 mV to +30 mV (when glutamate is present) or +80 mV (when arginine is present) (Foster 2004; Richard and Foster 2004). This flip in polarity of the membrane potential is thought to be the combined result of an increasing amount of protons in the cytoplasm as pH decreases and the decarboxylation products of the AR2 or AR3 pathways (Glu^- to GABA, Arg^+ to Agm^{2+}). When the acid stress is elevated, the membrane potential is restored with the help of the *E. coli* ClC chloride channel (Foster 2004; Gut et al. 2006) (Fig. 1).

The ClC channel is a $1H^+/2Cl^-$ antiporter, and its structure has been solved by X-ray crystallography (Dutzler et al. 2002) (Fig. 3). It is a homodimeric membrane protein in which each monomer can transport one Cl^- ion. The Cl^- selectivity filter is formed by Cl^- ion interacting with the dipoles of an α-helix and with nitrogen and hydroxyl groups (Dutzler et al. 2002). The ClC protein imports Cl^- to bring negative charges into the cell and simultaneously removes excess protons. This helps the cell to eventually recover a negative inner membrane potential (Foster 2004).

Potassium ions are also implicated in the regulation of cytoplasmic pH in *E. coli*. The K^+ influx systems including the Trk and Ktr K^+/H^+ symporters and K^+ channels play important roles in maintaining the electrochemical membrane potential and in regulating the cytoplasmic pH homeostasis (Epstein 2003). The requirement of K^+ ions depends on the external pH. White et al. (1992) showed that cells depleted of the K^+ transport systems can survive as well as WT at pH 8.0, but fail to survive at external pH of 6.0 when the concentration of K^+ is below 40 $mmol \cdot L^{-1}$.

Chaperone-based acid stress response

Proteins involved in the 3 enzyme-based pathways function mainly in the cytoplasm. However, *E. coli* also has acid resistance systems to protect periplasmic proteins, which are more vulnerable to acid denaturation and damage as the outer membrane porins are permeable to extracellular molecules. The small chaperones HdeA (9.7 kDa) and HdeB (9 kDa) are the primary players in the acid stress response in the periplasm (Gajiwala and Burley 2000) (Fig. 1). *hdeA* and *hdeB* are expressed from the same operon (Fig. 5). Although they only share 17% sequence identity, HdeA is thought to be structurally homologous to HdeB based on secondary structure prediction (Gajiwala and Burley 2000).

HdeA and HdeB are general chaperones that function in an ATP-independent manner. They recognize a variety of substrates and maintain them in a soluble state in the acidic environment. They can also form mixed aggregates with proteins that have failed to be solubilized (Malki et al. 2008) (Fig. 1). The presence of HdeA and HdeB was shown to decrease the size of protein aggregates in extreme acid (below pH 3); the chaperones also make these aggregates less hydrophobic. In vitro data shows that HdeA functions optimally below pH 3, while the optimal pH for HdeB is 3 (Kern et al. 2007). However, it seems that in vivo, both are required for optimal protection of periplasmic proteins at external pH of 2 to 3. Although in vitro they were shown to be able to help proteins refold once pH becomes neutral, it is possible that, in vivo, other enzymes such as periplasmic chaperones, disulfide isomerases, and (or) peptidyl prolyl isomerases may be involved (Malki et al. 2008).

The structure of HdeA has been solved by X-ray crystallography (Gajiwala and Burley 2000) (Fig. 3). HdeA is a compact single-domain protein with a hydrophobic core created by four α-helices. There is one disulfide bond formed between C18 and C66 that stabilizes the structure of the monomer. At neutral pH, HdeA exists in an inactive homodimeric form. The dimerization is mediated by interaction between hydrophobic residues, such as Val, Thr, and Ala from both monomers. When the pH is abruptly shifted below 3, HdeA dissociates into active monomers in a fraction of a second (Tapley et al. 2009). The monomer turns into a partially unfolded state that retains most of its secondary structure but not its tertiary structure (Tapley et al. 2009). The C18-C66 disulfide bond is essential for HdeA function by holding the protein together at this stage. The hydropho-

bic residues, previously at the dimer interface, are, consequently, exposed and are shown to adaptively interact with misfolded and (or) unfolded substrates to form different conformations of substrate-HdeA complexes (Tapley et al. 2009) (Fig. 1). The primary sequence of HdeA reveals an amphipathic property of this protein, in that the N- and C-termini of this protein are both positively charged and the middle region is hydrophobic (Wu et al. 2008). The N- and C-termini may help increase the solubility of the HdeA–substrate complexes at extreme acidic pH (Wu et al. 2008).

The mechanism of function of HdeB is proposed to be similar to that of HdeA. The only difference found so far is that the exposed surface of HdeB when it becomes disordered at pH 3 is less hydrophobic than that of HdeA (Kern et al. 2007). The function of HdeA/B at above pH 3 and below pH 7 has not been investigated. They function optimally at or below pH 3, mostly likely because the appropriate unfolding of these two chaperones only occurs under extreme acid conditions.

The *hdeA/B* genes are only induced in stationary phase cells at acidic pH. Regulators of *hdeA/B* include GadE, GadX, GadW, H-NS, LRP, and MarA. GadE activates the transcription of the *hdeA/B* genes chaperones under all conditions. GadX and GadW are both transcriptional repressors of *hdeA/B*. H-NS represses *hdeA/B* transcription at neutral pH (Malki et al. 2008). LRP, or leucine-response protein, is a strong repressor for *hdeA/B* transcription in minimal medium. MarA, an AraC/Xyls transcriptional regulator, represses *hdeA/B* transcription by increasing H-NS repression and interfering with GadE activation in stationary phase in the presence of sodium salicylate (Ruiz et al. 2008).

Recently, a cytoplasmic chaperone, Hsp31 (*hchA*), was implicated in acid resistance in *E. coli* (Mujacic and Baneyx 2007) (Fig. 1). Hsp31 is a heat-inducible homodimeric protein that belongs to the ThiI/DJ-1/PfpI superfamily (Figs. 2 and 3). It functions as a holdase that stabilizes unfolded protein intermediates until the elevation of stress. The transcription of *hchA* is induced by σ^s in stationary phase cells. Deletion of *hchA* results in markedly decreased activity of the AR2 and AR3 pathways. However, the mechanism of how this is achieved is not known (Mujacic and Baneyx 2007).

The acid fitness island in *E. coli*

Many of the regulatory genes for the Gad system (Fig. 4A), including *gadA/E/W/X*, as well as the chaperone and chaperone-related genes *hdeA/B/D*, are located as a cluster at position 3 651 984 – 3 665 603 bp on the chromosome (Fig. 5). This cluster is unique to *E. coli* and is termed the acid fitness island (Hommais et al. 2004).

Recently, more genes in this island were found to participate in acid resistance, including an outer membrane lipoprotein, Slp; a transcription regulator, YhiF; and two predicated membrane proteins, YhiD and HdeD. Slp and YhiF are required to protect cells against excreted toxic metabolites including the accumulated anions of dissociated weak acids after growth at low pH such as lactate, succinate, and formate. HdeD and YhiD are required for acid stress response when cells are grown at high density (>10^8 CFU·mL^{-1}) (Mates et al. 2007). Moreover, the transcription of these genes is activated by GadX and GadW, but it is unknown whether the activation is direct (Tucker et al. 2003).

Acid stress response in other enterobacteria

Although *E. coli* is the most commonly used model organism for studying acid resistance, it is important to also study other pathogenic bacteria to understand the differences in their acid resistance systems. Four other commonly studied enteropathogenic bacteria are *Vibrio cholera*, *Vibrio vulnifus*, *Shigella flexneri*, and *Salmonella typhimurium* (Table 1).

The two *Vibrio* species are intestinal pathogens. *Vibrio cholera* has an acid tolerance response (ATR) and an essential component of the ATR is the lysine decarboxylase system. *Vibrio cholera* CadA and CadB function similarly to the *E. coli* Cad system: the *cadA* and *cadB* genes are organized into an operon, and downstream of the *cadBA* operon lies the *cadC* gene. *Vibrio cholera* CadC is similar in sequence and function to the *E. coli* CadC. It specifically activates the transcription of *cadBA* operon under acid induction (pH 4.5). However, unlike *E. coli*, *V. cholera cadA* also possesses an independent promoter, so the *cadB* and *cadA* genes can also be transcribed monocistronically. The *cadBA* operon is transcribed constitutively at a low level independent of acid or CadC induction, although this basal level expression is insufficient for acid resistance (Merrell and Camilli 2000). In addition, a ClpB protein encoded by the *clpB1* gene in *V. cholera* was also implicated in the acid stress response (Nag et al. 2005). ClpB protein is a member of the Hsp100-family chaperone ATPases that function to dissolve protein aggregates.

Vibrio vulnifus causes food-borne gastroenteritis (Kim et al. 2006). It also encodes a Cad system, the expression of which is regulated by multiple factors. Because acid stress often induces superoxide stress, SoxR, a protein induced under superoxide stress, together with CadC, induces *cadBA* at low pH. The two activators bind at different regions on the same *cadBA* promoter: SoxR at –10 to –38 bp and CadC at approximately –233 bp from the transcription start of *cadBA* (Kim et al. 2006). The decarboxylation product, cadaverine, also helps to scavenge oxide radicals (Kim et al. 2006). AphB, a LysR family transcriptional regulator, indirectly induces the expression of *cadBA* by activating the transcription of *cadC*. The AphB homologue in *V. cholera*, however, functions in a virulence cascade instead of with CadC (Rhee et al. 2006). Lastly, LRP, the leucine responsive protein, cooperates with CadC to bind to the *cadBA* promoter and induce the expression of *cadBA* (Rhee et al. 2008).

So far, acid resistance pathways that permit survival in extreme acid (pH 2), such as the Gad and Arg systems, have not been found in the *Vibrio* species. Thus, the two *Vibrio* species are more acid sensitive than *E. coli* and *Shigella flexneri*.

Shigella flexneri has both the AR1 (the decarboxylase-independent pathway) and AR2 pathways (Bhagwat and Bhagwat 2004). However, there are several notable differences in AR2 of *Shigella flexneri* from that of *E. coli*. Expression of the *Shigella* Gad system requires acid stress and σ^s in stationary phase cells grown in minimal medium, whereas

Table 1. The decarboxylase systems in different enteric bacteria.

	Glutamate decarboxylase system			Arginine decarboxylase system			Lysine decarboxylase system			
	gadA/B	*gadC*	*gadE*	*adiA*	*adiC*	*adiY*	*cadA*	*cadB*	*cadC*	Reference
Escherichia coli	+	+	+	+	+	+	+	+	+	Foster (2004)
Vibrio cholera	–	–	–	–	–	–	+	+	+	Merrell and Camilli (2000)
Vibrio vulnifus	–	–	–	–	–	–	+	+	+	Kim et al. (2006)
Shigella flexneri	+	+	+	–	–	–	–	–	–	Bhagwat and Bhagwat (2004)
Salmonella typhimurium	–	–	–	+	+	+	+	+	+	Waterman and Small (1996); Kieboom and Abee (2006)

Note: +, gene present; –, gene not found.

the *E. coli* Gad system is induced in stationary phase regardless of medium (Bhagwat and Bhagwat 2004). In stationary phase *Shigella* cells, the expression level of *gadA*, *gadBC*, *gadE*, and *hdeA* genes decrease with increasing pH; *gadE* is not even transcribed at pH above 6.85. However, in *E. coli*, *gadE* transcripts can still be detected at pH 7.5 (Bhagwat and Bhagwat 2004). The *Shigella* Gad system is not induced in exponential phase cells (Waterman and Small 2003*a*). The σ^s-independent induction of the Gad system of *Shigella flexneri* requires a much stronger environmental signal, such as anaerobiosis and growth on glucose; in *E. coli*, the induction of the Gad system can happen under semi-aerobic conditions. In addition, HdeA is essential for the proper function of the Gad system in *Shigella flexneri* (Bhagwat and Bhagwat 2004).

Salmonella enterica serovar Typhimurium was previously thought unable to survive in extreme acid (below pH 3) because it lacks AR2 and AR3 systems. Although the AR2 pathway is absent in *Salmonella*, as indicated by Southern blotting against *Salmonella* DNA with a *gadC* probe, a functional arginine decarboxylase (AR3) system was recently discovered in this species (Waterman and Small 1996; Kieboom and Abee 2006). The *Salmonella* AR3 system is only induced when cells are grown in anoxic conditions (i.e., in the absence of oxygen); however, unlike in *E. coli*, the *adiY* gene in *Salmonella* is required for the proper activation of this system (Kieboom and Abee 2006).

Salmonella also has the AR4 lysine decarboxylase system. The *cadBA* operon is activated by CadC. However, unlike in *E. coli* where *cadC* is constitutively expressed, the *Salmonella enterica cadC* is induced by low pH and the presence of lysine. This could be explained by the observation that the promoters of the *cadC* gene in the two species have little sequence similarity (Lee et al. 2007).

Salmonella CadC has 3 domains, similar to that of *E .coli*: the N-terminal DNA-binding domain, the transmembrane domain, and the C-terminal periplasmic domain. At low pH and in the presence of lysine, CadC in *Salmonella* is cleaved in the vicinity of residue 210 located in a segment linking the transmembrane and periplasmic domains (Lee et al. 2008). This cleavage is proposed to then lead to the activation of the *cadBA* operon (Lee et al. 2008). The CadC proteins of *E. coli* and *Salmonella* share 58.4% sequence identity, and are predicted to have similar structures (Lee et al. 2007). However, it is not yet clear whether *E. coli* CadC undergoes a similar processing event to activate *cadBA* as *Salmonella* CadC.

Salmonella also has 2 other major acid tolerance response (ATR) systems. One of them is a log-phase ATR system. This system is induced when exponentially growing cells adapted at a moderate pH (4.5–5.8) undergo a transition to low pH (pH 3) (Audia et al. 2001). Over 60 acid shock proteins (ASPs) are produced during this response. These proteins include σ^s, Fur (the major iron regulator), Ada-DNA methyltransferase involved in DNA damage and repair, and the two-component PhoP/Q system. Protons prevent inhibition of PhoQ by Mg^{2+} by affecting its Mg^{2+} binding site. PhoQ can then phosphorylate PhoP to induce genes required for *Salmonella* to survive in macrophage phagolysosome (Audia et al. 2001). Importantly, this log-phase ATR also provides cross protection against other environmental challenges such as oxidative stress, heat shock, and high osmolarity, but not vice versa.

The other major ATR system in *Salmonella* is the stationary-phase ATR. It is induced by exposing stationary-phase cells to low pH (e.g., below pH 5) (Lee et al. 1994). Forty-eight ASPs are induced in this ATR and only 5 of them overlap with those induced in the log phase induced acid tolerance (Audia et al. 2001). This system is σ^s-independent and is not affected by mutation in genes such as *fur* and *phoP*. One example of genes induced by this response is OmpR, which is part of the EnvZ/OmpR 2-component system. Two known genes induced by OmpR are the OmpC and OmpF porins. Upon acid stress, OmpR is activated by phosphorylation from the phosphate donor acetyl phosphate instead by EnvZ. Although OmpR is required for optimal function of the stationary-phase ATR, how it is induced and what OmpR-dependent genes are involved in acid tolerance are not known (Bang et al. 2000). The *Salmonella* CadC is a mild repressor of *ompR* transcription. In fact, unlike in *E. coli* and *Vibrio*, the *Salmonella* CadC regulates many genes apart from the *cadBA* operon. Proteins down regulated by *cadC* include proteins involved in glycolysis (PfkA, PfkB, FbaB, and STM4519), energy production (AtpD), and stress response (Tig and HslU). Proteins up-regulated by CadC include outer membrane proteins (OmpC and OmpF). However, it is not clear whether the CadC regulation is direct or indirect (Lee et al. 2007).

The *hdeA/B* genes are absent from the *Salmonella* genome, as confirmed by Southern blotting (Waterman and Small 1996). Other chaperones that might be involved in

the acid stress response of *Salmonella* have not yet been identified.

Concluding remarks

The discussion presented on the acid resistance systems in 5 enteropathogenic bacteria demonstrates that acid stress response is an extremely complex process. Mediators of this process encompass decarboxylases, chaperones, small noncoding RNAs, and protein factors involved in a variety of cellular functions such as DNA topology modulation, superoxide stress, ion concentration responses, and cellular transport. Nevertheless, despite the overwhelmingly complicated regulation network, common core acid resistance systems, notably the glutamate, arginine, and lysine decarboxylase systems, are shared by these model organisms. The complex machinery of acid stress response can be seen as a sign of the absolute necessity for this response to initiate accurately and function properly for cell survival. Extensive research is still needed to understand the complexity of regulation and function of the bacterial acid stress response at the molecular level. This information will be tremendously valuable for developing antibiotics that are simultaneously effective against multiple species of bacteria.

Acknowledgements

The authors thank Mr. Usheer Kanjee and Dr. Majida El Bakkouri for helpful comments and suggestions. B.Z. is the recipient of a National Sciences and Engineering Research Council of Canada Undergraduate Student Research Award. This work was supported by a grant from the Canadian Institutes of Health Research (MOP-67210) to W.A.H.

References

Andréll, J., Hicks, M.G., Palmer, T., Carpenter, E.P., Iwata, S., and Maher, M.J. 2009. Crystal structure of the acid-induced arginine decarboxylase from Escherichia coli: reversible decamer assembly controls enzyme activity. Biochemistry, **48**(18): 3915–3927. doi:10.1021/bi900075d. PMID:19298070.

Audia, J.P., Webb, C.C., and Foster, J.W. 2001. Breaking through the acid barrier: an orchestrated response to proton stress by enteric bacteria. Int. J. Med. Microbiol. **291**(2): 97–106. doi:10.1078/1438-4221-00106. PMID:11437344.

Bang, I.S., Kim, B.H., Foster, J.W., and Park, Y.K. 2000. OmpR regulates the stationary-phase acid tolerance response of Salmonella enterica serovar typhimurium. J. Bacteriol. **182**(8): 2245–2252. doi:10.1128/JB.182.8.2245-2252.2000. PMID:10735868.

Bearson, S., Bearson, B., and Foster, J.W. 1997. Acid stress responses in enterobacteria. FEMS Microbiol. Lett. **147**(2): 173–180. doi:10.1111/j.1574-6968.1997.tb10238.x. PMID:9119190.

Bhagwat, A.A., and Bhagwat, M. 2004. Comparative analysis of transcriptional regulatory elements of glutamate-dependent acid-resistance systems of Shigella flexneri and Escherichia coli O157:H7. FEMS Microbiol. Lett. **234**(1): 139–147. doi:10.1111/j.1574-6968.2004.tb09525.x. PMID:15109732.

Bordi, C., Théraulaz, L., Méjean, V., and Jourlin-Castelli, C. 2003. Anticipating an alkaline stress through the Tor phosphorelay system in Escherichia coli. Mol. Microbiol. **48**(1): 211–223. doi:10.1046/j.1365-2958.2003.03428.x. PMID:12657056.

Capitani, G., De Biase, D., Aurizi, C., Gut, H., Bossa, F., and Grütter, M.G. 2003. Crystal structure and functional analysis of Escherichia coli glutamate decarboxylase. EMBO J. **22**(16): 4027–4037. doi:10.1093/emboj/cdg403. PMID:12912902.

Castanie-Cornet, M.P., and Foster, J.W. 2001. Escherichia coli acid resistance: cAMP receptor protein and a 20 bp cis-acting sequence control pH and stationary phase expression of the gadA and gadBC glutamate decarboxylase genes. Microbiology, **147**(Pt 3): 709–715. PMID:11238978.

Dell, C.L., Neely, M.N., and Olson, E.R. 1994. Altered pH and lysine signalling mutants of cadC, a gene encoding a membrane-bound transcriptional activator of the Escherichia coli cadBA operon. Mol. Microbiol. **14**(1): 7–16. doi:10.1111/j.1365-2958.1994.tb01262.x. PMID:7830562.

Dutyshev, D.I., Darii, E.L., Fomenkova, N.P., Pechik, I.V., Polyakov, K.M., Nikonov, S.V., et al. 2005. Structure of *Escherichia coli* glutamate decarboxylase (GADα) in complex with glutarate at 2.05 Å resolution. Acta Cryst. **D61**: 230–235. doi:10.1107/S0907444904032147. PMID:15735332.

Dutzler, R., Campbell, E.B., Cadene, M., Chait, B.T., and MacKinnon, R. 2002. X-ray structure of a ClC chloride channel at 3.0 A reveals the molecular basis of anion selectivity. Nature, **415**(6869): 287–294. doi:10.1038/415287a. PMID:11796999.

Epstein, W. 2003. The roles and regulation of potassium in bacteria. Prog. Nucleic Acid Res. Mol. Biol. **75**: 293–320. doi:10.1016/S0079-6603(03)75008-9. PMID:14604015.

Fang, Y., Jayaram, H., Shane, T., Kolmakova-Partensky, L., Wu, F., Williams, C., et al. 2009. Structure of a prokaryotic virtual proton pump at 3.2 A resolution. Nature, **460**(7258): 1040–1043. PMID:19578361.

Foster, J.W. 2004. Escherichia coli acid resistance: tales of an amateur acidophile. Nat. Rev. Microbiol. **2**(11): 898–907. doi:10.1038/nrmicro1021. PMID:15494746.

Gajiwala, K.S., and Burley, S.K. 2000. HDEA, a periplasmic protein that supports acid resistance in pathogenic enteric bacteria. J. Mol. Biol. **295**(3): 605–612. doi:10.1006/jmbi.1999.3347. PMID:10623550.

Gao, X., Lu, F., Zhou, L., Dang, S., Sun, L., Li, X., et al. 2009. Structure and mechanism of an amino acid antiporter. Science, **324**(5934): 1565–1568. doi:10.1126/science.1173654. PMID:19478139.

Giangrossi, M., Zattoni, S., Tramonti, A., De Biase, D., and Falconi, M. 2005. Antagonistic role of H-NS and GadX in the regulation of the glutamate decarboxylase-dependent acid resistance system in Escherichia coli. J. Biol. Chem. **280**(22): 21498–21505. doi:10.1074/jbc.M413255200. PMID:15795232.

Gong, S., Ma, Z., and Foster, J.W. 2004. The Era-like GTPase TrmE conditionally activates gadE and glutamate-dependent acid resistance in Escherichia coli. Mol. Microbiol. **54**(4): 948–961. doi:10.1111/j.1365-2958.2004.04312.x. PMID:15522079.

Gut, H., Pennacchietti, E., John, R.A., Bossa, F., Capitani, G., De Biase, D., and Grütter, M.G. 2006. Escherichia coli acid resistance: pH-sensing, activation by chloride and autoinhibition in GadB. EMBO J. **25**(11): 2643–2651. doi:10.1038/sj.emboj.7601107. PMID:16675957.

Hansen, A.M., Qiu, Y., Yeh, N., Blattner, F.R., Durfee, T., and Jin, D.J. 2005. SspA is required for acid resistance in stationary phase by downregulation of H-NS in Escherichia coli. Mol. Microbiol. **56**(3): 719–734. doi:10.1111/j.1365-2958.2005.04567.x. PMID:15819627.

Heuveling, J., Possling, A., and Hengge, R. 2008. A role for Lon protease in the control of the acid resistance genes of Escherichia coli. Mol. Microbiol. **69**(2): 534–547. doi:10.1111/j.1365-2958.2008.06306.x. PMID:18630346.

Hommais, F., Krin, E., Coppée, J.Y., Lacroix, C., Yeramian, E., Danchin, A., and Bertin, P. 2004. GadE (YhiE): a novel activa-

tor involved in the response to acid environment in Escherichia coli. Microbiology, **150**: 61–72. doi:10.1099/mic.0.26659-0. PMID:14702398.

Iyer, R., Williams, C., and Miller, C. 2003. Arginine-agmatine antiporter in extreme acid resistance in Escherichia coli. J. Bacteriol. **185**(22): 6556–6561. doi:10.1128/JB.185.22.6556-6561.2003. PMID:14594828.

Jeong, K.C., Hung, K.F., Baumler, D.J., Byrd, J.J., and Kaspar, C.W. 2008. Acid stress damage of DNA is prevented by Dps binding in Escherichia coli O157:H7. BMC Microbiol. **8**(1): 181. doi:10.1186/1471-2180-8-181. PMID:18922164.

Jin, Y., Watt, R.M., Danchin, A., and Huang, J.D. 2009. Small noncoding RNA GcvB is a novel regulator of acid resistance in Escherichia coli. BMC Genomics, **10**(1): 165. doi:10.1186/1471-2164-10-165. PMID:19379489.

Kashiwagi, K., Miyamoto, S., Suzuki, F., Kobayashi, H., and Igarashi, K. 1992. Excretion of putrescine by the putrescine-ornithine antiporter encoded by the potE gene of Escherichia coli. Proc. Natl. Acad. Sci. U.S.A. **89**(10): 4529–4533. doi:10.1073/pnas.89.10.4529. PMID:1584788.

Kern, R., Malki, A., Abdallah, J., Tagourti, J., and Richarme, G. 2007. Escherichia coli HdeB is an acid stress chaperone. J. Bacteriol. **189**(2): 603–610. doi:10.1128/JB.01522-06. PMID:17085547.

Kieboom, J., and Abee, T. 2006. Arginine-dependent acid resistance in Salmonella enterica serovar Typhimurium. J. Bacteriol. **188**(15): 5650–5653. doi:10.1128/JB.00323-06. PMID:16855258.

Kim, J.S., Choi, S.H., and Lee, J.K. 2006. Lysine decarboxylase expression by Vibrio vulnificus is induced by SoxR in response to superoxide stress. J. Bacteriol. **188**(24): 8586–8592. doi:10.1128/JB.01084-06. PMID:17012399.

Lease, R.A., Smith, D., McDonough, K., and Belfort, M. 2004. The small noncoding DsrA RNA is an acid resistance regulator in Escherichia coli. J. Bacteriol. **186**(18): 6179–6185. doi:10.1128/JB.186.18.6179-6185.2004. PMID:15342588.

Lee, I.S., Slonczewski, J.L., and Foster, J.W. 1994. A low-pH-inducible, stationary-phase acid tolerance response in Salmonella typhimurium. J. Bacteriol. **176**(5): 1422–1426. PMID:8113183.

Lee, Y.H., Kim, B.H., Kim, J.H., Yoon, W.S., Bang, S.H., and Park, Y.K. 2007. CadC has a global translational effect during acid adaptation in Salmonella enterica serovar Typhimurium. J. Bacteriol. **189**(6): 2417–2425. doi:10.1128/JB.01277-06. PMID:17209022.

Lee, Y.H., Kim, J.H., Bang, I.S., and Park, Y.K. 2008. The membrane-bound transcriptional regulator CadC is activated by proteolytic cleavage in response to acid stress. J. Bacteriol. **190**(14): 5120–5126. doi:10.1128/JB.00012-08. PMID:18487329.

Ma, Z., Richard, H., Tucker, D.L., Conway, T., and Foster, J.W. 2002. Collaborative regulation of Escherichia coli glutamate-dependent acid resistance by two AraC-like regulators, GadX and GadW (YhiW). J. Bacteriol. **184**(24): 7001–7012. doi:10.1128/JB.184.24.7001-7012.2002. PMID:12446650.

Ma, Z., Gong, S., Richard, H., Tucker, D.L., Conway, T., and Foster, J.W. 2003. GadE (YhiE) activates glutamate decarboxylase-dependent acid resistance in Escherichia coli K-12. Mol. Microbiol. **49**(5): 1309–1320. doi:10.1046/j.1365-2958.2003.03633.x. PMID:12940989.

Ma, Z., Masuda, N., and Foster, J.W. 2004. Characterization of EvgAS-YdeO-GadE branched regulatory circuit governing glutamate-dependent acid resistance in Escherichia coli. J. Bacteriol. **186**(21): 7378–7389. doi:10.1128/JB.186.21.7378-7389.2004. PMID:15489450.

Malki, A., Le, H.T., Milles, S., Kern, R., Caldas, T., Abdallah, J., and Richarme, G. 2008. Solubilization of protein aggregates by the acid stress chaperones HdeA and HdeB. J. Biol. Chem. **283**(20): 13679–13687. doi:10.1074/jbc.M800869200. PMID:18359765.

Mates, A.K., Sayed, A.K., and Foster, J.W. 2007. Products of the Escherichia coli acid fitness island attenuate metabolite stress at extremely low pH and mediate a cell density-dependent acid resistance. J. Bacteriol. **189**(7): 2759–2768. doi:10.1128/JB.01490-06. PMID:17259322.

Merrell, D.S., and Camilli, A. 2000. Regulation of vibrio cholerae genes required for acid tolerance by a member of the "ToxR-like" family of transcriptional regulators. J. Bacteriol. **182**(19): 5342–5350. doi:10.1128/JB.182.19.5342-5350.2000. PMID:10986235.

Momany, C., Ernst, S., Ghosh, R., Chang, N.L., and Hackert, M.L. 1995. Crystallographic structure of a PLP-dependent ornithine decarboxylase from Lactobacillus 30a to 3.0 A resolution. J. Mol. Biol. **252**(5): 643–655. doi:10.1006/jmbi.1995.0526. PMID:7563080.

Mujacic, M., and Baneyx, F. 2007. Chaperone Hsp31 contributes to acid resistance in stationary-phase Escherichia coli. Appl. Environ. Microbiol. **73**(3): 1014–1018. doi:10.1128/AEM.02429-06. PMID:17158627.

Nag, S., Das, S., and Chaudhuri, K. 2005. In vivo induced clpB1 gene of Vibrio cholerae is involved in different stress responses and affects in vivo cholera toxin production. Biochem. Biophys. Res. Commun. **331**(4): 1365–1373. doi:10.1016/j.bbrc.2005.04.052. PMID:15883026.

Neely, M.N., and Olson, E.R. 1996. Kinetics of expression of the Escherichia coli cad operon as a function of pH and lysine. J. Bacteriol. **178**(18): 5522–5528. PMID:8808945.

Neely, M.N., Dell, C.L., and Olson, E.R. 1994. Roles of LysP and CadC in mediating the lysine requirement for acid induction of the Escherichia coli cad operon. J. Bacteriol. **176**(11): 3278–3285. PMID:8195083.

Opdyke, J.A., Kang, J.G., and Storz, G. 2004. GadY, a small-RNA regulator of acid response genes in Escherichia coli. J. Bacteriol. **186**(20): 6698–6705. doi:10.1128/JB.186.20.6698-6705.2004. PMID:15466020.

Rhee, J.E., Jeong, H.G., Lee, J.H., and Choi, S.H. 2006. AphB influences acid tolerance of Vibrio vulnificus by activating expression of the positive regulator CadC. J. Bacteriol. **188**(18): 6490–6497. doi:10.1128/JB.00533-06. PMID:16952939.

Rhee, J.E., Kim, K.S., and Choi, S.H. 2008. Activation of the Vibrio vulnificus cadBA Operon by Leucine-responsive regulatory protein is mediated by CadC. J. Microbiol. Biotechnol. **18**(11): 1755–1761. PMID:19047817.

Richard, H., and Foster, J.W. 2004. Escherichia coli glutamate- and arginine-dependent acid resistance systems increase internal pH and reverse transmembrane potential. J. Bacteriol. **186**(18): 6032–6041. doi:10.1128/JB.186.18.6032-6041.2004. PMID:15342572.

Richard, H., and Foster, J.W. 2007. Sodium regulates Escherichia coli acid resistance, and influences GadX- and GadW-dependent activation of gadE. Microbiology, **153**: 3154–3161. doi:10.1099/mic.0.2007/007575-0. PMID:17768258.

Ruiz, C., McMurry, L.M., and Levy, S.B. 2008. Role of the multidrug resistance regulator MarA in global regulation of the hdeAB acid resistance operon in Escherichia coli. J. Bacteriol. **190**(4): 1290–1297. doi:10.1128/JB.01729-07. PMID:18083817.

Sayed, A.K., and Foster, J.W. 2009. A 750 bp sensory integration region directs global control of the Escherichia coli GadE acid

resistance regulator. Mol. Microbiol. **71**(6): 1435–1450. doi:10.1111/j.1365-2958.2009.06614.x. PMID:19220752.

Sayed, A.K., Odom, C., and Foster, J.W. 2007. The Escherichia coli AraC-family regulators GadX and GadW activate gadE, the central activator of glutamate-dependent acid resistance. Microbiology, **153**: 2584–2592. doi:10.1099/mic.0.2007/007005-0. PMID:17660422.

Shi, X., and Bennett, G.N. 1994. Effects of rpoA and cysB mutations on acid induction of biodegradative arginine decarboxylase in Escherichia coli. J. Bacteriol. **176**(22): 7017–7023. PMID: 7961466.

Snider, J., Gutsche, I., Lin, M., Baby, S., Cox, B., Butland, G., et al. 2006. Formation of a distinctive complex between the inducible bacterial lysine decarboxylase and a novel AAA+ ATPase. J. Biol. Chem. **281**(3): 1532–1546. doi:10.1074/jbc.M511172200. PMID:16301313.

Soksawatmaekhin, W., Kuraishi, A., Sakata, K., Kashiwagi, K., and Igarashi, K. 2004. Excretion and uptake of cadaverine by CadB and its physiological functions in Escherichia coli. Mol. Microbiol. **51**(5): 1401–1412. doi:10.1046/j.1365-2958.2003.03913.x. PMID:14982633.

Soksawatmaekhin, W., Uemura, T., Fukiwake, N., Kashiwagi, K., and Igarashi, K. 2006. Identification of the cadaverine recognition site on the cadaverine-lysine antiporter CadB. J. Biol. Chem. **281**(39): 29213–29220. doi:10.1074/jbc.M600754200. PMID:16877381.

Stewart, N., Feng, J., Liu, X., Chaudhuri, D., Foster, J.W., Drolet, M., and Tse-Dinh, Y.C. 2005. Loss of topoisomerase I function affects the RpoS-dependent and GAD systems of acid resistance in Escherichia coli. Microbiology, **151**: 2783–2791. doi:10.1099/mic.0.28022-0. PMID:16079354.

Takada, A., Umitsuki, G., Nagai, K., and Wachi, M. 2007. RNase E is required for induction of the glutamate-dependent acid resistance system in Escherichia coli. Biosci. Biotechnol. Biochem. **71**(1): 158–164. doi:10.1271/bbb.60423. PMID:17213667.

Tapley, T.L., Körner, J.L., Barge, M.T., Hupfeld, J., Schauerte, J.A., Gafni, A., et al. 2009. Structural plasticity of an acid-activated chaperone allows promiscuous substrate binding. Proc. Natl. Acad. Sci. U.S.A. **106**(14): 5557–5562. doi:10.1073/pnas.0811811106. PMID:19321422.

Tetsch, L., Koller, C., Haneburger, I., and Jung, K. 2008. The membrane-integrated transcriptional activator CadC of Escherichia coli senses lysine indirectly via the interaction with the lysine permease LysP. Mol. Microbiol. **67**(3): 570–583. doi:10.1111/j.1365-2958.2007.06070.x. PMID:18086202.

Tramonti, A., De Canio, M., Delany, I., Scarlato, V., and De Biase, D. 2006. Mechanisms of transcription activation exerted by GadX and GadW at the gadA and gadBC gene promoters of the glutamate-based acid resistance system in Escherichia coli. J. Bacteriol. **188**(23): 8118–8127. doi:10.1128/JB.01044-06. PMID:16980449.

Tucker, D.L., Tucker, N., Ma, Z., Foster, J.W., Miranda, R.L., Cohen, P.S., and Conway, T. 2003. Genes of the GadX-GadW regulon in Escherichia coli. J. Bacteriol. **185**(10): 3190–3201. doi:10.1128/JB.185.10.3190-3201.2003. PMID:12730179.

Waterman, S.R., and Small, P.L. 1996. Identification of sigma S-dependent genes associated with the stationary-phase acid-resistance phenotype of Shigella flexneri. Mol. Microbiol. **21**(5): 925–940. doi:10.1046/j.1365-2958.1996.00058.x. PMID: 8885264.

Waterman, S.R., and Small, P.L. 2003*a*. Identification of the promoter regions and sigma(*s*)-dependent regulation of the gadA and gadBC genes associated with glutamate-dependent acid resistance in Shigella flexneri. FEMS Microbiol. Lett. **225**(1): 155–160. doi:10.1016/S0378-1097(03)00508-1. PMID: 12900035.

Waterman, S.R., and Small, P.L. 2003*b*. Transcriptional expression of Escherichia coli glutamate-dependent acid resistance genes gadA and gadBC in an hns rpoS mutant. J. Bacteriol. **185**(15): 4644–4647. doi:10.1128/JB.185.15.4644-4647.2003. PMID: 12867478.

Watson, N., Dunyak, D.S., Rosey, E.L., Slonczewski, J.L., and Olson, E.R. 1992. Identification of elements involved in transcriptional regulation of the Escherichia coli cad operon by external pH. J. Bacteriol. **174**(2): 530–540. PMID:1370290.

White, S., Tuttle, F.E., Blankenhorn, D., Dosch, D.C., and Slonczewski, J.L. 1992. pH dependence and gene structure of inaA in Escherichia coli. J. Bacteriol. **174**(5): 1537–1543. PMID: 1537798.

Wu, Y.E., Hong, W., Liu, C., Zhang, L., and Chang, Z. 2008. Conserved amphiphilic feature is essential for periplasmic chaperone HdeA to support acid resistance in enteric bacteria. Biochem. J. **412**(2): 389–397. doi:10.1042/BJ20071682. PMID:18271752.

Zhao, Y., Liu, D., Kaluarachchi, W.D., Bellamy, H.D., White, M.A., and Fox, R.O. 2003. The crystal structure of Escherichia coli heat shock protein YedU reveals three potential catalytic active sites. Protein Sci. **12**(10): 2303–2311. doi:10.1110/ps.03121403. PMID:14500888.

Zwir, I., Shin, D., Kato, A., Nishino, K., Latifi, T., Solomon, F., et al. 2005. Dissecting the PhoP regulatory network of Escherichia coli and Salmonella enterica. Proc. Natl. Acad. Sci. U.S.A. **102**(8): 2862–2867. doi:10.1073/pnas.0408238102. PMID: 15703297.