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MicroReview

A multifaceted role for polyamines in bacterial pathogens

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Summary

Polyamines are polycationic molecules with a hydrocarbon backbone and multiple amino groups. Descriptions of the physiological roles of polyamines have often been limited to their interaction with negatively charged nucleic acids. Of late, reports linking polyamines to microbial carcinogenesis, biofilm formation, escape from phagolysosomes, bacteriocin production, toxin activity and protection from oxidative and acid stress have been published, providing insights about their other important but lesser known functions. This review focuses on recently discovered novel functions of polyamines in microorganisms, with an emphasis on bacterial pathogens of humans.

Introduction

Microbial pathogens face diverse environmental stresses during growth in a human host and have consequently acquired multiple adaptations to survive and multiply in difficult conditions. Microbial physiology can change substantially during growth *in vivo*, and acquisition of scarce but essential nutrients seems to be a critical cell function. Mechanisms used by microbial pathogens to obtain small molecule metabolites during colonization and infection of a host have generally been overlooked due to the prevailing emphasis on more traditional virulence factors. A more comprehensive view of bacterial metabolism and physiology in a host will lead to a deeper understanding of microbial pathogenesis.

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Polyamines are small aliphatic hydrocarbon molecules with quaternary nitrogen groups that have a net positive charge at physiological pH. Efficient DNA replication, transcription and translation are essential for a pathogen to colonize and multiply successfully in its host. Organic polycationic molecules such as polyamines and inorganic cations such as magnesium and calcium play a crucial role in maintaining optimal conformation of negatively charged nucleic acids. In bacteria, the processes of polyamine uptake, synthesis and degradation are co-ordinated to regulate stringently intracellular polyamine levels. Our understanding of polyamines and their role in growth and virulence in human pathogens has recently increased substantially. Polyamines now appear to be more than trivial organic molecules whose functions are interchangeable or even dispensable. This review summarizes the recent findings on the contribution of polyamines, polyamine biosynthesis and transport systems to the pathogenesis and virulence of important human bacterial pathogens.

Polyamine metabolism in bacteria

Polyamines such as putrescine (1,4-diaminobutane) and cadaverine (1,5-diaminopentane) are primary diamines with two amino groups each, while spermidine [*N*-(3-aminopropyl)butane-1,4-diamine] and spermine [*N*,*N*′-bis(3-aminopropyl)butane-1,4-diamine] contain three and four amino groups respectively (Fig. 1). Unlike cations such as Mg^{+2} and Ca^{+2}, the positive charges on polyamines are found at regularly spaced intervals along flexible hydrocarbon chains. Because of their unique charge-structure conformation, polyamines can serve as electrostatic bridges between negative phosphate charges on nucleic acids and other negatively charged polymers.

Putrescine, spermidine, spermine and cadaverine are the most widely distributed cellular polyamines and are essential for normal cellular growth and multiplication of both prokaryotic and eukaryotic cells (Cohen, 1997). The intracellular content of spermidine (1–3 mM) is higher than that of putrescine (0.1–0.2 mM) in almost all bacteria, although putrescine (10–30 mM) is the predominant polyamine in *Escherichia coli*, followed by spermidine

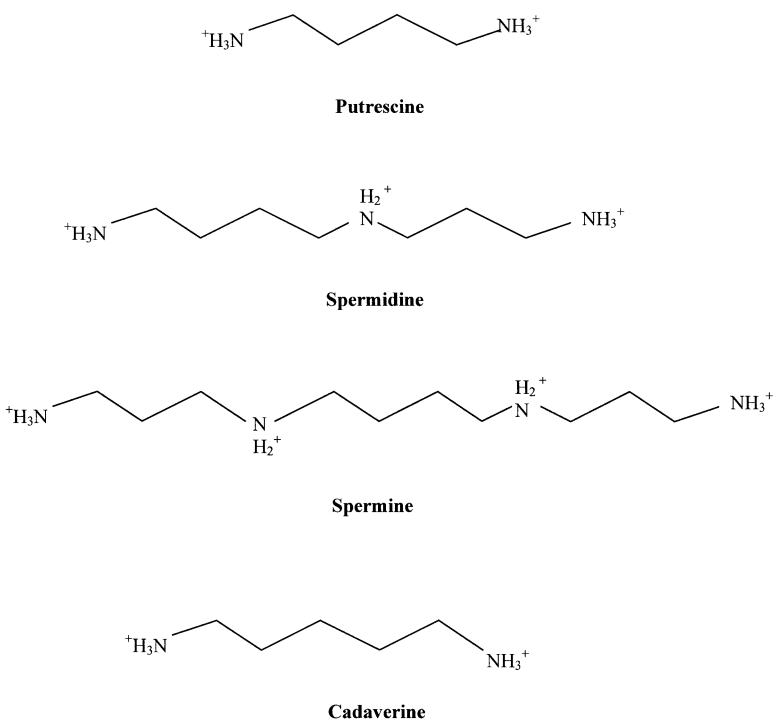

Fig. 1. Structures of common microbial polyamines.

(1–3 mM) (Cohen, 1997). The presence of spermine in bacterial cells is not well established. In *E. coli*, an important model for polyamine research, spermine is not synthesized *de novo* but exogenous spermine can satisfy cellular polyamine requirements under laboratory conditions (Dubin and Rosenthal, 1960). In almost all other pathogenic bacteria, spermine is only found in cells when exogenous spermine is present in the medium. Cadaverine is the least prevalent of all naturally occurring bacterial polyamines and is normally absent in *E. coli*. However, *E. coli* synthesizes cadaverine during anaerobic growth in the presence of its precursor amino acid lysine at a low pH (Watson *et al*., 1992) or in the absence of putrescine biosynthesis (Cohen, 1997).

Bacterial polyamine synthesis usually commences with the decarboxylation of precursor amino acids directly into polyamines or other intermediates, which are subsequently modified to yield functional polyamines. In *E. coli* and many *Pseudomonas* species, putrescine is synthesized via two pathways: (i) decarboxylation of ornithine to putrescine by ornithine decarboxylase (ODC) encoded by the *speC* gene, and (ii) arginine decarboxylation to agmatine by arginine decarboxylase (*speA*), followed by the conversion of agmatine to putrescine and urea by agmatine ureohydrolase (*speB*) (Tabor and Tabor, 1985; Cohen, 1997) (Fig. 2). *Pseudomonas aeruginosa* has an additional pathway in which agmatine is converted to putrescine via *N*-carbamoylputrescine (Nakada and Itoh, 2003).

The key enzyme of the putrescine biosynthesis pathway, ODC, is regulated by multiple mechanisms. ODC is reversibly inhibited by either putrescine (Morris and Fillingame, 1974) or spermidine (Cohen, 1997). ODC activity is also regulated at the transcription level by cyclic AMP (cAMP) and cAMP receptor protein complex, which repress *speC* expression in *E. coli* (Wright *et al*., 1986). An antizyme has also been shown to reduce ODC activity in *E. coli* and some other bacteria (Lioliou and Kyriakidis, 2004), whereas GTP binding to ODC enhances enzyme activity (Oliveira *et al*., 1997). Arginine decarboxylase activity is inhibited by elevated levels of intracellular spermidine and putrescine in a manner similar to ODC (Moore and Boyle, 1991).

Spermidine biosynthesis genes *speE* and *speD* are organized as an operon and are distant from the *speABC* genes that are involved in putrescine biosynthesis (Xie *et al*., 1989). Spermidine biosynthesis in *E. coli* commences with the conversion of methionine into S-adenosyl methionine (SAM) by the enzyme methionine adenosyltransferase (SAM synthetase) encoded by *metK* (Fig. 2). Transcription of *metK* is under the control of a *trans*-acting regulatory element encoded by *metJ* (Saint-Girons *et al*., 1986). The enzyme SAM decarboxylase (*speD*) then converts SAM into decarboxylated SAM, which acts as a cofactor for spermidine biosynthesis (Tabor *et al*., 1986). Finally, spermidine synthase (SpeE) converts putrescine to spermdine in the presence of decarboxylated SAM. The crystal structure of spermidine

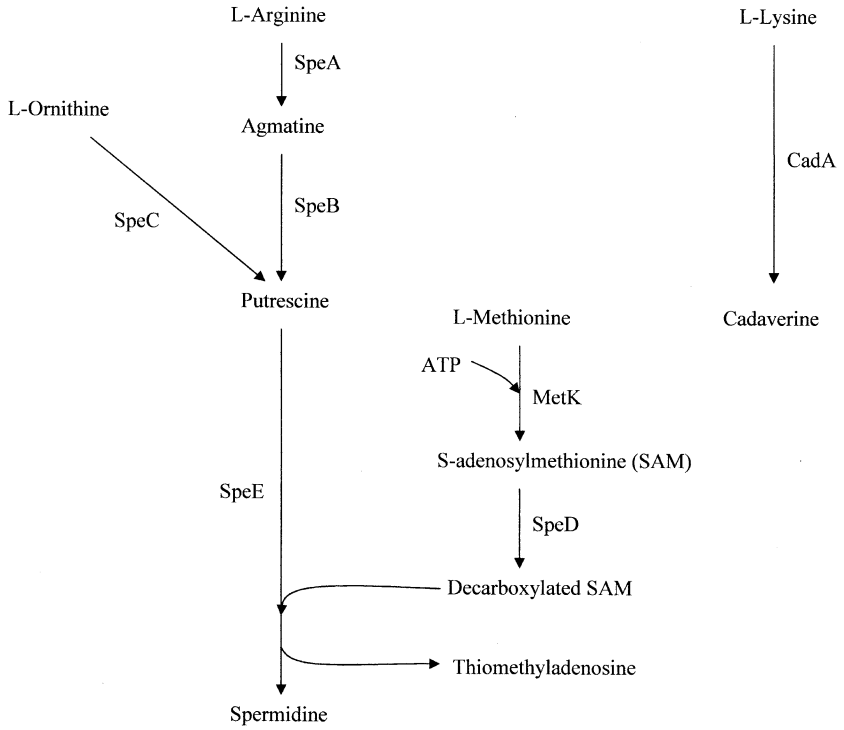

Fig. 2. Biosynthetic pathways for polyamines in *E. coli*. Cadaverine synthesis occurs predominantly when lysine is abundant in the growth medium or putrescine synthesis is blocked. Assigned enzyme designations: SpeA – arginine decarboxylase, SpeB – agmatine ureohydrolase, SpeC – ornithine decarboxylase, SpeD – SAM decarboxylase, SpeE – spermidine synthase, MetK – methionine adenosyltransferase, CadA – lysine decarboxylase.

synthase from the human pathogen *Helicobacter pylori* has been reported (Lu *et al*., 2007). Spermidine inhibits SAM decarboxylase activity in *E. coli*, implying that spermidine is a major regulator of this enzyme (Kashiwagi and Igarashi, 1988). Interestingly, others have reported that exogenous spermidine reduces expression of *E. coli* SAM decarboxylase in the presence of a plasmid encoding rat SAM decarboxylase (Salmikangas *et al*., 1989). In *Bacillus subtilis*, spermidine inhibits SAM decarboxylase expression in cells in stationary phase (Sekowska *et al*., 2000). Overall, spermidine seems to be a key regulator of bacterial SAM decarboxylase activity and expression.

Deletion in either the *speE* or *speD* genes in *E. coli* has minimal effect on its growth rate, indicating that spermidine is not an essential polyamine for *E. coli* growth (Tabor *et al*., 1983). Interestingly, *E. coli* mutated in *speA*, *B* and *C*, but with a functional *speD* gene, synthesizes little spermidine but can synthesize tetra-amines like spermine (Hafner *et al*., 1977). Thus, spermine biosynthesis occurs in the face of deficiency of other more commonly occurring polyamines in *E. coli*.

Polyamine transport systems

In addition to *de novo* polyamine synthesis, bacteria have transport systems that allow uptake of extracellular polyamines. Polyamine transporters have primarily been characterized in *E. coli* but analysis of microbial genome sequences indicates that they are likely to be present in many Gram-positive and Gram-negative human pathogens as well as in archaea, yeast and protozoa. This high degree of conservation suggests that these systems provide a significant adaptive and/or survival advantage to microorganisms (Table 1).

Escherichia coli polyamine transport systems (Pot) include two ABC (ATP-binding cassette) transporters that are selective for either putrescine or spermidine. Additionally, two antiporters, one that exchanges putrescine for ornithine (PotE) and one that allows exchange of lysine and cadaverine (CadB), as well as uniporters for putrescine and cadaverine uptake have also been described. The order of preference for extracellular polyamines in *E. coli* is: putrescine > spermidine > spermine (Kashiwagi *et al*., 1986).

Polyamine ABC transporter genes in *E. coli* and many other human pathogens are organized as four-gene operons and designated as *potABCD* (spermidine uptake) and *potFGHI* (putrescine uptake). In *E. coli*, PotD and PotF are periplasmic substrate-binding proteins that bind extracellular polyamines, and PotA and PotG proteins are membrane-associated cytosolic ATPases. The remaining proteins have membrane-spanning α-helices and form transmembrane channels for polyamine transport (Kashiwagi *et al*., 1990; Pistocchi *et al*., 1993). Binding of spermidine to PotD leads to a conformation change, resulting in a more compact protein structure. This complex triggers a conformation change in PotBC that results in a structural change in PotA that leads to ATP

Table 1. Polyamine transport systems in representative human bacterial pathogens.

Organism	Polyamine transporter	Reference
Bacillus anthracis	PotABCD	Read *et al.* (2003)
Escherichia coli	PotABCD; PotFGHI; PotE	Igarashi and Kashiwagi (1999)
Haemophilus influenzae	PotABCD; PotE	Fleischmann *et al.* (1995)
Legionella pneumophila	PotABCD	Chien *et al.* (2004)
Mycoplasma pneumoniae	PotABC	Himmelreich *et al.* (1996)
Neisseria gonorrhoeae	PotABCD	GenBank Accession No. AE004969
Neisseria meningitidis	PotABCD	Tettelin *et al.* (2000)
Pseudomonas aeruginosa	PotABCD; PotFGHI	Stover *et al.* (2000)
Salmonella enterica paratyphi	PotABCD; PotFGHI; PotE	McClelland *et al.* (2004)
Salmonella enterica typhi	PotABCD; PotFGHI; PotE	Deng *et al.* (2003)
Salmonella typhimurium	PotABCD; PotFGHI; PotE	McClelland *et al.* (2001)
Shigella boydii, Shigella sonnei	PotABCD; PotFGHI; PotE	Yang *et al.* (2005)
Shigella dysenteria	PotABCD; PotFGHI	Yang *et al.* (2005)
Shigella flexneri	PotABCD; PotFGHI; PotE	Wei *et al.* (2003)
Staphylococcus aureus	PotABCD	Baba *et al.* (2002)
Staphylococcus epidermidis	PotABCD	Gill *et al.* (2005)
Staphylococcus haemolyticus	PotABCD	Takeuchi *et al.* (2005)
Streptococcus mutans	PotABCD	Ajdic *et al.* (2002)
Streptococcus pneumoniae	PotABCD	Tettelin *et al.* (2001)
Streptococcus pyogenes	PotABCD	Beres *et al.* (2002)
Treponema pallidum	PotABCD	Machius *et al.* (2007)
Vibrio cholerae	PotABCD; PotE	Heidelberg *et al.* (2000)
Yersinia pestis	PotABCD; PotFGHI	Deng *et al.* (2002)

hydrolysis and spermidine uptake. Non-polar mutations in any of the genes in the *potABCD* operon abolish polyamine transport, indicating that all four gene products are essential (Furuchi *et al.*, 1991). In *E. coli*, spermidine can also bind to PotA and decrease its ATPase activity with a resultant inhibition of polyamine uptake (Igarashi and Kashiwagi, 2006). PotD can also downregulate transcription of the *potABCD* operon (Antognoni *et al.*, 1999). Thus, both receptor and ligand can function as feedback regulators of polyamine transport. The crystal structure of PotD from *E. coli* indicates that it has two β-α-β domains with a spermidine-binding cleft in the middle (Sugiyama *et al.*, 1996). *E. coli* PotD binds spermidine with a dissociation constant of 3.2 μM, and can also bind to putrescine, albeit with a higher K_d of 100 μM (Kashiwagi *et al.*, 1993). The crystal structure of PotF revealed a binding site for putrescine, but unlike PotD it does not bind spermidine as it lacks additional amino acid residues in its binding site to accommodate spermidine. Similarly, binding assays with PotF show that it possesses a high binding affinity to only putrescine ($K_d = 2.0$ μM) and does not bind other polyamines (Vassylyev *et al.*, 1998).

In *Streptococcus pneumoniae* (pneumococcus), a polycistronic mRNA molecule encoding the *potABCD* genes is cotranscribed with the *murB* gene involved in peptidoglycan biosynthesis (Ware *et al.*, 2005). The pneumococcal *potABCD* genes bear a high degree of sequence identity to the corresponding genes in *E. coli*, and a proposed model for the polyamine transporter is very similar (Fig. 3). Pneumococcal PotD has a typical Gram-positive leader sequence and is predicted to be a secreted protein. However, it does not have a readily identifiable surface anchor mechanism typical of Gram-positive surface proteins. Work in our laboratory has shown that PotD co-purifies with membrane fractions and that it is likely to be a lipoprotein (Shah *et al.*, 2006), although the precise mechanism for lipidation is unknown and is currently under study.

PotE and the cadaverine transporter CadB each possess 12 transmembrane segments with both amino-

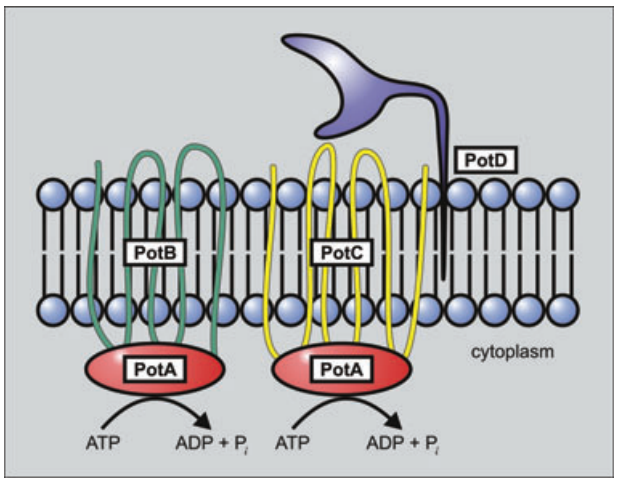

Fig. 3. Proposed structure of a polyamine transporter in *Streptococcus pneumoniae*. PotA – cytoplasmic protein with ATP-binding motif that couples ATP hydrolysis to translocation of polyamines; PotBC – two polypeptides, with five or six membrane-spanning α-helical hydrophobic domains; PotD – extracellular substrate-binding protein.

and carboxy-termini located in the cytoplasm. These membrane proteins represent a different class of transporters that are capable of both uptake and extrusion. Polyamine uptake without extrusion, mediated by PotE or CadB, is dependent on membrane potential alone. In *E. coli*, extrusion of putrescine by PotE is coupled to ornithine import and, likewise, CadB couples cadaverine excretion to lysine uptake (Kashiwagi *et al*., 1997; Soksawatmaekhin *et al*., 2004).

Genes for lysine decarboxylase (*cadA*) and a lysine-cadaverine antiporter (*cadB*) form the *cadBA* operon in *E. coli*. This operon is induced in response to acidic pH, resulting in synthesis of cadaverine by CadA, followed by the excretion of cadaverine, uptake of lysine by CadB and neutralization of the medium (Soksawatmaekhin *et al*., 2004). The *cadC* gene, which is separated from *cadBA*, encodes a 58 kDa inner membrane protein CadC. CadC binds to operator sites Cad1 and Cad2 upstream of the *cadBA* promoter region and acts as a transcriptional activator of the *cadBA* operon, whereas LysP and H-NS repress *cadBA* expression under non-inducing conditions (Shi *et al*., 1993; Neely *et al*., 1994). In a proposed model for transcriptional regulation of the *cadBA* operon, H-NS is involved in the formation of a repression complex under non-inducing conditions. This complex is dissociated by binding of CadC to Cad1 under inducing conditions, while CadC binding to Cad2 activates the expression of the *cad* operon (Kuper and Jung, 2005).

Interaction with nucleic acids

Intracellular polyamines are primarily found as complexes with RNA and work in conjunction with Mg^{+2} to stabilize higher orders of structure. In fact, polyamines can accelerate *in vitro* protein synthesis compared with Mg^{+2} alone. Polyamine binding to RNA molecules might cause unique structural changes that are different from those induced by magnesium (Igarashi *et al*., 1974). Polyamines can also bind to ribosomes and increase accuracy of codon usage during protein synthesis (Ito and Igarashi, 1986), facilitate translational read-through of mRNAs with a UAA stop codon (Higashi *et al*., 2006) and increase the efficiency of nonsense mutation suppression in T4 phage using a tRNA containing an amber mutation (Tabor and Tabor, 1982). Supplemental putrescine can restore virulence gene expression in *Shigella flexneri* mutants that are unable to synthesize modified nucleosides necessary for tRNA synthesis. It is possible that a direct interaction between putrescine and virulence gene mRNAs results in more efficient translation and expression of these virulence genes (Durand and Bjork, 2003).

In addition, translation of the mRNA for OppA, a periplasmic substrate-binding protein of the *E. coli* oligopeptide uptake system, is enhanced by the binding of spermidine to a GC-rich double-stranded region near the Shine–Dalgarno (SD) sequence of the OppA mRNA. This interaction causes a structural change in the SD sequence leading to an interaction between the AUG codon and 30S ribosomal subunit, allowing the formation of an initiation complex (Yoshida *et al*., 1999). A similar mechanism is observed in polyamine-mediated translational enhancement of a transcription regulator (FecI) of an iron transporter operon and a transcriptional regulator (Fis) of rRNA, tRNA synthetases and adenylate cyclase (Yoshida *et al*., 2001; Yoshida *et al*., 2004). The translation of RpoN, H-NS and Cra mRNA is more efficient when *E. coli* polyamine auxotrophic mutants are supplemented with polyamines. RpoN and H-NS mRNA transcripts have unusual SD sequences, while the Cra mRNA has an inefficient initiation codon (GUG) (Terui *et al*., 2007). Overall, polyamine binding appears to cause a conformational change in a stretch of relatively unstable double-stranded RNA containing the SD sequence and the initiation codon, leading to enhancement of translation.

Surface structures and polyamines

Polyamines play a crucial role in outer membrane functions, primarily by their effects on the production and function of porins (Dela Vega and Delcour, 1996). Porins are homo-trimeric transmembrane channels that allow the diffusion of hydrophilic compounds across the outer membrane of Gram-negative cells. Porins OmpC and OmpF in *E. coli* interact with polyamines in a concentration- and a transmembrane voltage-dependent manner (Iyer and Delcour, 1997). Putrescine and spermidine bind to aspartic acid residues of OmpF and OmpC, altering the charge and pore size, resulting in channel closure and subsequently decreasing outer membrane permeability (Iyer *et al*., 2000).

Streptococcus pneumoniae requires the amino alcohol choline for synthesis of teichoic and lipoteichoic acids. Choline anchors proteins that play important roles in pneumococcal virulence. Putrescine can substitute for choline *in vitro* during pneumococcal growth in a choline-deficient environment (Ware *et al*., 2005). However, incorporation of putrescine in the pneumococcal cell wall results in the loss of surface-bound choline-binding proteins, suggesting that polyamine substitution would attenuate virulence. Putrescine is also a constituent of the outer membrane in some Gram-negative bacteria like *Salmonella enterica* SP Typhimurium, *E. coli* and *Proteus mirabilis* (Koski and Vaara, 1991; Vinogradov and Perry, 2000), while, cadaverine is linked to the peptidoglycan of *Veillonella alcalescens* (Kamio, 1987) and covalently attached to the D-glutamic acid residues in a peptide containing diaminopimelic acid in *Selenomonas ruminatum* (Kamio *et al*., 1986). Although polyamines are prima-

rily considered intracellular constituents, accumulating evidence indicates their importance in maintaining surface structures in bacteria.

Response to physiological stress

Microbial pathogens face oxidative stress *in vivo* that induces an adaptive response involving production of proteins with protective functions. Reactive oxygen species cause double-strand breaks and base modifications in DNA sequences with high GC content. Superoxide dismutase (SOD) is a key enzyme that protects cellular nucleic acids from the damaging effects of superoxide radicals. Spermine and spermidine function as free radical scavengers and, in conjunction with SOD, reduce DNA strand breakage by oxygen radicals (Khan *et al*., 1992a, b; Ha *et al*., 1998). Concentrations of oxygen that are non-toxic to wild-type *E. coli* cells are lethal to polyamine-deficient mutants (Chattopadhyay *et al*., 2003). Polyamines therefore seem to play an important adjunctive role in protecting cells from the toxic effects of reactive oxygen species.

On the other hand, exposure of a polyamine-deficient mutant to paraquat results in increased expression of *soxS*, which codes for a key regulator of the response to oxidative stress in *E. coli*, while addition of exogenous putrescine to the mutant leads to a decrease in *soxS* expression (Jung and Kim, 2003b). In addition, another oxidative stress response regulator SoxR regulates the *cadBA* operon in *Vibrio vulnificus* in a CadC-independent manner. SoxR binds to the promoter region of the *cadBA* operon, resulting in increased synthesis of cadaverine, which scavenges superoxide radicals and increases tolerance to oxidative stress and also reduces SOD expression (Kim *et al*., 2006). Furthermore, pneumococcal *potD* transcription increases in response to oxidative and temperature stress, choline limitation and more importantly during murine bacteraemia (P. Shah and E. Swiatlo, submitted). Polyamine uptake from the environment may potentially help pneumococci to survive various host microenvironments. Thus, polyamine-mediated protection against oxidative stress in bacteria might involve direct binding of polyamines and nucleic acids, scavenging of the reactive oxygen species or differential expression of protective enzymes like SOD. All of these mechanisms most likely contribute to variable degrees, with the relative contribution depending, for example, on the metabolic state of the cell, growth medium and the type of oxidative insult.

Survival in an acidic environment under nutrient limitation is a key requirement for intestinal pathogens and commensals. Polyamines seem to be key mediators in the response to acid stress in bacteria. In *E. coli*, the *gadA* and *gadB* genes encode glutamate decarboxylase, which is induced in an acidic environment and contributes to acid resistance. Expression of *gadA* and *gadB* is regulated by polyamines via reduction in intracellular cAMP levels; polyamine-deficient mutants fail to produce glutamate decarboxylase and show greatly reduced survival in an acidic medium (Jung and Kim, 2003a). The viability of *E. coli* cells starved of inorganic phosphate (P_i) is severely reduced in either aerobic or anaerobic conditions. Addition of lysine to a P_i-limited medium induces expression of the *cadBA* operon. Expression of lysine decarboxylase, in conjunction with glutamate decarboxylase, increases the pH of the medium and most likely allows the cells to tolerate higher levels of formate produced during anaerobic, P_i-limited conditions (Moreau, 2007). A similar mechanism for acid resistance is seen in *Salmonella*, where both lysine decarboxylase and RpoS-dependent acid-shock proteins are required for survival at low pH (Park *et al*., 1996). It was recently shown that the *cadBA* operon in *S. enterica*, and *ompF* and *ompC* gene transcription regulator OmpR, are regulated by CadC in response to low pH (Lee *et al*., 2007). Overall, the induction of amino acid decarboxylases like CadA in response to acid stress might facilitate survival of microbial pathogens *in vivo*.

Polyamines and microbial pathogenesis

Role of cadaverine biosynthesis in escape from phagolysosomes and decreased adherence

Assessment of polyamine biosynthesis in bacterial pathogens is often limited to a few well-characterized pathways primarily in the context of physiology. Likewise, characterization of polyamine transport systems is mostly restricted to evaluating their role in response to physiological stress. This focus seems to be changing, as a number of recently published reports have recognized polyamines, polyamine biosynthesis and transport mechanisms as 'classical' virulence factors involved in multiple aspects of bacterial pathogenesis.

The human pathogen *Shigella* is closely related to *E. coli* and the four common *Shigella* species associated with humans are thought to have arisen from a common *E. coli* progenitor strain. These genetic exchanges often result in acquisition of virulence determinants that are absent in less pathogenic ancestors. In some cases, loss of genes is also associated with increased virulence. The *cadA* gene is present in most *E. coli* strains but absent in *Shigella*. Expression of *cadA* in *Shigella* results in the inhibition of enterotoxin activity. Pre-treatment of eukaryotic cells with cadaverine makes them less susceptible to the effects of *Shigella* enterotoxins (Maurelli *et al*., 1998). This may occur through polyamine interactions with cell membranes or toxin receptors, or alterations in intracellular signalling cascades. However, polyamines may perhaps also directly interact with enterotoxin in a manner

similar to polyamine inactivation of anthrax lethal factor (Goldman *et al*., 2006).

Cadaverine also inhibits lysis of phagosomes by *S. flexneri* and prevents bacterial dissemination within the intestinal epithelium. Mechanistic details about the role of cadaverine in phagolysosome stability are not known. Enhancement of the stability of the phagolysosome membrane or interaction with components critical to the formation and maintenance of the phagolysosomal vacuole have been offered as possible explanations (Fernandez *et al*., 2001). It has become evident that cadaverine has an important role in the pathogenesis of *Shigella* infections and further work remains to examine the role of polyamines and other intracellular pathogens.

The *cad* operon is absent in certain Shiga toxin-producing *E. coli* strains. Restoration of lysine decarboxylase activity leads to reduced production of intimin, an adhesin required for the formation of attaching and effacing lesions in toxigenic strains (Torres *et al*., 2005). Cadaverine may be an inhibitor of *E. coli* adherence, however, exogenous cadaverine does not significantly reduce adherence of *cad* deficient strains. Some aspect of *in situ* cadaverine synthesis other than end-product potentially exerts a strong negative effect on intimin synthesis and the adherence phenotype.

Microbial carcinogenesis and polyamines

Polyamine biosynthesis in eukaryotic cells is controlled mainly by the activity of ODC and SAM decarboxylase. Uncontrolled ODC expression and polyamine biosynthesis lead to malignant transformation and cancer in certain human cells (Gerner and Meyskens, 2004). Targeting polyamine metabolism in tumour cells and other hyperproliferative diseases might be a promising therapeutic option (Casero and Marton, 2007). Gastric infection by *H. pylori* is considered to be an important risk factor for gastric carcinoma (El-Omar *et al*., 2000). Polyamines are key effector molecules of carcinoma caused by *H. pylori* infection. ODC activity is upregulated in *H. pylori*-infected cells, and eradication of the infection leads to decreased host cell ODC activity (Alam *et al*., 1994). Elevated ODC activity from *Helicobacter* infection may be a marker of premalignant lesions (Patchett *et al*., 1996).

Multiple mechanisms are involved in dysregulation of ODC by *H. pylori*. Upregulation of ODC activity by bacterial lipopolysacchride (LPS) is one potential mechanism contributing to *H. pylori*-induced carcinoma. Bacterial LPS is a potent mitogen that activates a variety of immune responses. LPS binds to CD14, which is a co-receptor with Toll-like receptor 4 for LPS and is present on the surfaces of mononuclear cells neutrophils (Mai *et al*., 1991). *Helicobacter* LPS appears to facilitate tumour progression in enterochromaffin-like cells by activating ODC via CD14 (Kidd *et al*., 2000). However, it is important to note that, under most circumstances, spermine inhibits pro-inflammatory cytokines in human mononuclear cells in response to LPS stimulation (Zhang *et al*., 1999). Thus, polyamines can stimulate uncontrolled proliferation in transformed cells as well as participate in counter-regulatory mechanisms against monocyte activation in injured and infected tissues.

Host cell apoptosis and polyamines

Helicobacter infection also causes activation of neutrophils, monocytes and macrophages; however, these responses are ineffective in preventing disease. *H. pylori* decrease macrophage survival by modulating host cell polyamine biosynthesis. Transcription of *c-myc* is upregulated in *H. pylori*-infected macrophages. c-Myc binds to the ODC promoter region resulting in elevated ODC expression and macrophage apoptosis. Conversely, inhibition of either ODC or c-Myc binding to the ODC promoter region reduces apoptosis of infected cells (Cheng *et al*., 2005).

In eukaryotic cells polyamines are oxidized by polyamine oxidase (PAOh1) that converts spermine to spermidine without the intermediate acetylation step catalysed by spermine N1-acetyltransferase (SSAT). Oxidation of spermine results in production of H_2O_2, which can cause apoptosis by mitochondrial membrane depolarization (Vujcic *et al*., 2002). *Helicobacter* induces expression of PAOh1 in a mouse model of infection. Reduction of PAOh1 expression by siRNA resulted in significant reduction in H_2O_2 production and *Helicobacter*-induced apoptosis, while overexpression of PAOh1 resulted in enhanced H_2O_2 production, membrane depolarization and caspase activation leading to apoptosis (Chaturvedi *et al*., 2004; Xu *et al*., 2004).

Other microbial pathogens also disrupt host cell polyamine metabolism during infection. Bronchoalveolar lavage (BAL) fluid from animals infected with *Pneumocystis jiroveci* contains high levels of spermidine, N1-acetylspermine and N1-acetylspermidine which are thought be derived from *P. jiroveci* metabolism. This BAL fluid can induce apoptosis of alveolar macrophages (Lasbury *et al*., 2007). Similarly, polyamines derived primarily from bacterial metabolism are present in high concentrations in human gingival crevicular fluid from inflamed periodontal pockets (Lamster *et al*., 1987). Oral crevicular polymorphonuclear leukocyte cells (PMN) play a crucial role in preventing bacterial invasion of periodontal tissue (Miyasaki, 1991). Spermine, spermidine and, to a lesser extent, putrescine have all been shown to accelerate PMN apoptosis in a dose-dependent manner (Mariggio *et al*., 2004). Other reports have implicated polyamines found in gingival fluid in facilitating granule

release, respiratory burst (Walters and Chapman, 1995) and impairment of chemotaxis (Walters *et al*., 1995).

Polyamines and innate defence mechanisms

Inducible nitric oxide synthase (iNOS)-derived nitric oxide (NO) is a key effector in the innate immune response. *H. pylori* upregulates iNOS production in gastric mucosa but does not necessarily result in increased NO production in *H. pylori*-infected macrophages (Fu *et al*., 1999). *Helicobacter* uses two strategies to counter the increase in host cell iNOS production. First, *H. pylori* arginase decreases NO levels by competing with host cell iNOS for the common substrate L-arginine (Gobert *et al*., 2001). Second, *Helicobacter* induces arginase II as well as ODC activity in infected macrophages. Arginase II converts L-arginine to L-ornithine which is converted to spermine and spermidine (Gobert *et al*., 2002). Interestingly, spermine synthesized by the arginase II/ODC pathway also inhibits iNOS translation. Knockdown of ODC expression with siRNA in macrophages infected with *H. pylori* results in a significant increase in iNOS production and enhanced bacterial killing. On the other hand, exogenous spermine attenuates iNOS translation and, consequently, NO production; furthermore, it decreases killing in a concentration-dependent manner (Bussiere *et al*., 2005). This relationship between ODC, spermine and iNOS represents a unique feature of *H. pylori* pathogenesis.

Polyamine transporters as vaccine antigens

Components of polyamine ABC transporters also seem to be promising vaccine antigens. *S. pneumoniae* has a *potABCD* operon with a high degree of similarity to that found in *E. coli* (Ware *et al*., 2005). A functional polyamine transporter appears to be necessary for full expression of pneumococcal virulence (Polissi *et al*., 1998; Ware *et al*., 2006). Systemic immunization with recombinant PotD elicits a protective antibody response against otherwise fatal pneumonia and bacteraemia in a mouse model (Shah and Swiatlo, 2006). In addition, mucosal immunizations with PotD reduce nasopharyngeal colonization of *S. pneumoniae* clinical isolates in mice (P. Shah and E. Swiatlo unpubl. results). These data suggest that PotD might be effective as a component of a protein-based pneumococcal vaccine. Similarly, immunization with recombinant PotF protects mice against lethal challenge with *Burkholderia pseudomallei* (Harland *et al*., 2007).

Effects on biofilm formation and phenotypes

Polyamines have been implicated in the control of biofilm formation in several human pathogens. In *Yersinia pestis*, mutations in either *speA* or *speC* reduced attachment to a solid surface yet had minimal effect on biofilm thickness. A *speA-speC* double mutant, however, was incapable of producing any detectable biofilm and had a markedly reduced intracellular putrescine level. Addition of putrescine restored biofilm production in a dose-dependent manner. Surprisingly, complementation of the *speA-speC* double mutant with a wild-type *speA* allele alone was sufficient for biofilm formation (Patel *et al*., 2006).

Norspermidine, which contains one carbon less than spermidine, is the most prevalent polyamine in *Vibrio* species. The *mbaA* (maintenance of biofilm architecture) gene is part of an operon that also encodes a norspermidine sensor, NspS, and a hypothetical cytoplasmic protein in *Vibrio cholerae* (Karatan *et al*., 2005). MbaA, a putative integral membrane protein, is thought to have periplasmic domains and function as a regulator of biofilm formation. Increasing environmental concentrations of norspermidine activates biofilm formation in *V. cholerae* in the presence of NspS and MbaA. Deletion of *nspS* shows reduction in transcription of genes involved in exopolysaccharide synthesis and biofilm formation. Norspermidine binding to NspS can purportedly change the nature of its interaction with the periplasmic domains of MbaA and derepress genes involved in biofilm formation. Further investigations of the interactions between NspS and MbaA, as well as NspS and norspermidine, will help define the role of environmental polyamine sensing in *Vibrio* biofilm formation.

The swarming phenotype of *P. mirabilis* is usually linked to the expression of virulence genes such as haemolysin, IgA protease and urease (Allison *et al*., 1992). Transposon inactivation of *speAB* genes in *P. mirabilis* leads to a loss of the swarming phenotype that can be reversed by exogenous putrescine (Sturgill and Rather, 2004). The mechanisms by which putrescine regulates swarming are not known. Because of their pleiotropic effects on transcription and translation polyamines may affect the global expression of myriad regulatory proteins controlling the swarming phenotype.

Biosynthesis of molecules involved in pathogenesis

Spermidine is used in the synthesis of the siderophore petrobactin by *Bacillus anthracis*. Petrobactin plays an important role in iron acquisition and virulence, and does not bind to siderocalin, a host protein that binds bacterial siderophores (Oves-Costales *et al*., 2007). Vibriobactin and vulnibactin, iron-scavenging molecules of *V. cholerae* and *V. vulnificus*, contain norspermidine, suggesting an essential role for this polyamine in iron-limited environments (Okujo *et al*., 1994; Keating *et al*., 2000).

Colicins are a family of protein toxins produced by *E. coli* that kill other susceptible *E. coli* cells. In response

to the colicin-ColE7 treatment, increase in polyamine biosynthesis, as well as expression of PotD and OppA, is observed in *E. coli*. Conversely, mutants deficient in putrescine and spermidine biosynthesis show significant reduction in ColE7 production that can be restored by addition of polyamines to the medium. These results indicate that polyamines play an important role in ColE7 production. Polyamine-deficient mutants are also more susceptible to ColE7. Finally, in wild-type *E. coli*, exposure to polyamines reduces expression of TolA, BtuB, OmpF and OmpC proteins that are involved in colicin uptake (Pan *et al*., 2006). Taken together these observations suggest that polyamines stimulate colicin production and possibly downregulate proteins essential for colicin uptake simultaneously, conferring a survival advantage on colicin producing cells implying an essential role for polyamines in toxin immunity.

Conclusion

Polyamines constitute a ubiquitous family of small molecules that have diverse functions in both eukaryotic and prokaryotic cellular physiology. Their role in the pathogenesis of bacterial infections has often been overlooked but recent experimental evidence emphasizes the importance of polyamines in several key aspects of pathogenesis, survival and virulence of many human microbial pathogens. The contribution of polyamines to the natural history of bacterial infections is blurring the traditional dichotomy between bacterial physiology and virulence. As information on polyamine metabolism in bacterial pathogens continues to expand, potentially novel targets for prevention or treatment of infectious diseases will undoubtedly emerge.

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