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Mini review

Polyamines: Emerging players in bacteria–host interactions

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ABSTRACT

Polyamines are small polycationic molecules found in almost all cells and associated with a wide variety of physiological processes. In recent years it has become increasingly clear that, in addition to core physiological functions, polyamines play a crucial role in bacterial pathogenesis. Considerable evidence has built up that bacteria have evolved mechanisms to turn these molecules to their own advantage and a novel standpoint to look at host–bacterium interactions emerges from the interplay among polyamines, host cells and infecting bacteria. In this review, we highlight how human bacterial pathogens have developed their own resourceful strategies to exploit polyamines or manipulate polyamine-related processes to optimize their fitness within the host. Besides contributing to a better understanding of the complex relationship between a pathogen and its host, acquisitions in this field have a significant potential towards the development of novel antibacterial therapeutic approaches.

Introduction

Polyamines are small polycationic molecules associated, in eukaryotic as well as in prokaryotic cells, with a broad range of biological functions: translation, gene regulation, stress resistance, cell proliferation and differentiation (Igarashi and Kashiwagi, 2010a). In bacteria, putrescine, cadaverine, spermidine and spermine are the predominant polyamines (Fig. 1). Their intracellular content is regulated by concerted biosynthesis and uptake mechanisms, as well as by degradation and efflux processes (Tabor and Tabor, 1985).

Synthesis of bacterial spermidine, putrescine and cadaverine usually depends on the decarboxylation of precursor aminoacids or other intermediates which are then converted into functional polyamines (Fig. 2A). In addition to the *de novo* synthesis pathways, specific transport systems often exist which allow the uptake of polyamines from the external environment. These systems are widespread in bacteria and very well conserved among both, Gram negative and Gram positive microorganisms. In *Escherichia coli*, the best known case so far, polyamine uptake relies on two ABC transporters that are specific for either putrescine or spermidine, and on uniporters operating on putrescine and cavaderine. Traffic of polyamines with the external environment is facilitated by two antiporters, exchanging putrescine for ornithine and lysine for cadaverine (Igarashi and Kashiwagi, 2010b)

In recent years it has become increasingly evident that, in addition to core physiological functions, polyamines are crucial also to the virulence phenotype of many bacterial pathogens (Shah and Swiatlo, 2008). Numerous and diverse strategies are adopted by bacterial pathogens, directly involving polyamines or proteins implicated in the polyamine biosynthesis and transport pathways. In this review, we will describe how in several life threatening human bacterial pathogens polyamines contribute to optimize virulence or survival within the host.

Shigella: the yin-yang role of polyamines in the invasion process

The essential role played by polyamines in bacterial virulence is nicely exemplified by *Shigella*, an intracellular pathogen which belongs to the *E. coli* species and causes a severe enteric syndrome in humans (Sansonetti, 2006). Shigellosis is endemic throughout the world and millions of cases occur every year, mostly in the developing countries. The pathogenicity of *Shigella* strains is based on their capacity to reach and invade colonic epithelial cells, leading to intracellular bacterial multiplication and spread to adjacent cells with consequent cell death and destruction of the colonic mucosa (Sansonetti, 2006) (Fig. 3). The cellular pathogenesis of shigellosis is the sum of the complex action of a large number of bacterial virulence factors, mainly located on a large virulence plasmid (pINV)

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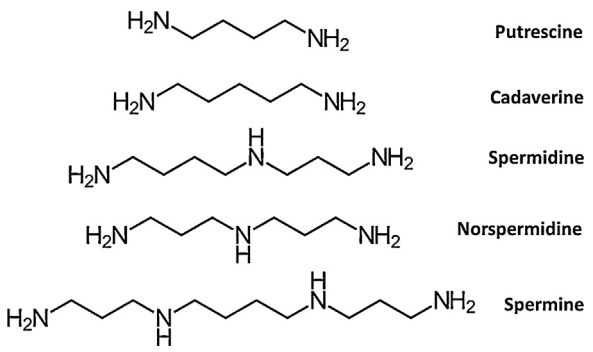

Fig. 1. Major polyamines associated with bacterial pathogenesis.

(Parsot, 2005). Full expression of pINV virulence genes is achieved by a delicate interplay among global and specific regulators (Falconi et al., 2001; Prosseda et al., 2002, 2004), and sRNAs molecules (Giangrossi et al., 2010; Tran et al., 2011).

While the acquisition of pINV is regarded as one of the most critical events towards pathogenicity, a significant complementary step has been the emergence of mutations in anti-virulence genes (Prosseda et al., 2007, 2012; Bliven and Maurelli, 2012). These so-called pathoadaptive mutations have led to the inactivation of several chromosomal genes which negatively interfere with the expression of the *Shigella* virulence phenotype. In particular, they have caused a drastic change in the polyamine content of *Shigella* as compared to *E. coli*, its commensal ancestor (Maurelli et al., 1998; Casalino et al., 2010; Barbagallo et al., 2011). Indeed, as a result of different mutations or deletions in the *cad* locus, cadaverine is lost from all *Shigella* species (Maurelli et al., 1998; Day et al., 2001; Casalino et al., 2005). The silencing of the *cad* genes is crucial in the optimization of the pathogenicity process in *Shigella*, since cadaverine protects the colonic mucosa from the adverse affects of the ShET1/ShET2 enterotoxins and negatively affects *Shigella*-induced proinflammatory events by inhibiting PMN migration to the infection site. Cadaverine also stabilizes the endosomal membrane, blocking the release of *Shigella* into the cytoplasm of infected host cells (Bliven and Maurelli, 2012; Maurelli et al., 1998) (Fig. 3).

As opposed to cadaverine loss, *Shigella* exhibits marked spermidine accumulation due to the lack of spermidine acetyltransferase (SAT), the enzyme encoded by the *speG* gene, which converts spermidine into its inert form, acetylspermidine. In analogy to the lack of the *cad* genes, also the loss of *speG* functionality is a case of pathoadaptive mutation obtained through convergent evolution (Barbagallo et al., 2011). Higher spermidine levels correlate with increased survival in response to oxidative stress, such as the exposure to hydrogen peroxide experienced by the bacterium during the infection of macrophages (Fig. 3). As one of the first steps of *Shigella* infection, survival within macrophages is crucial for successful invasion (Sansonetti, 2006). It is not surprising that during pathoadaptation, *Shigella* has altered its polyamine profile to improve resistance to the hostile conditions of the macrophage environment. The high level of spermidine stimulates the expression of OxyR and this, in turn, promotes the expression of the *katG* gene, which encodes a hydroperoxidase that contributes to increase bacterial antioxidant defences.

Besides spermidine and cadaverine, also putrescine is involved in the virulence of *Shigella*. Indeed, the addition of putrescine can restore virulence in *Shigella flexneri* mutants that are unable to synthesize modified nucleotides required for RNA synthesis (Durand and Björk, 2003). Moreover, putrescine has also been shown to relieve the ornithine repression exerted on *Shigella* virulence in minimal medium (Durand and Björk, 2009). All together the observations available so far on the involvement of polyamines in the

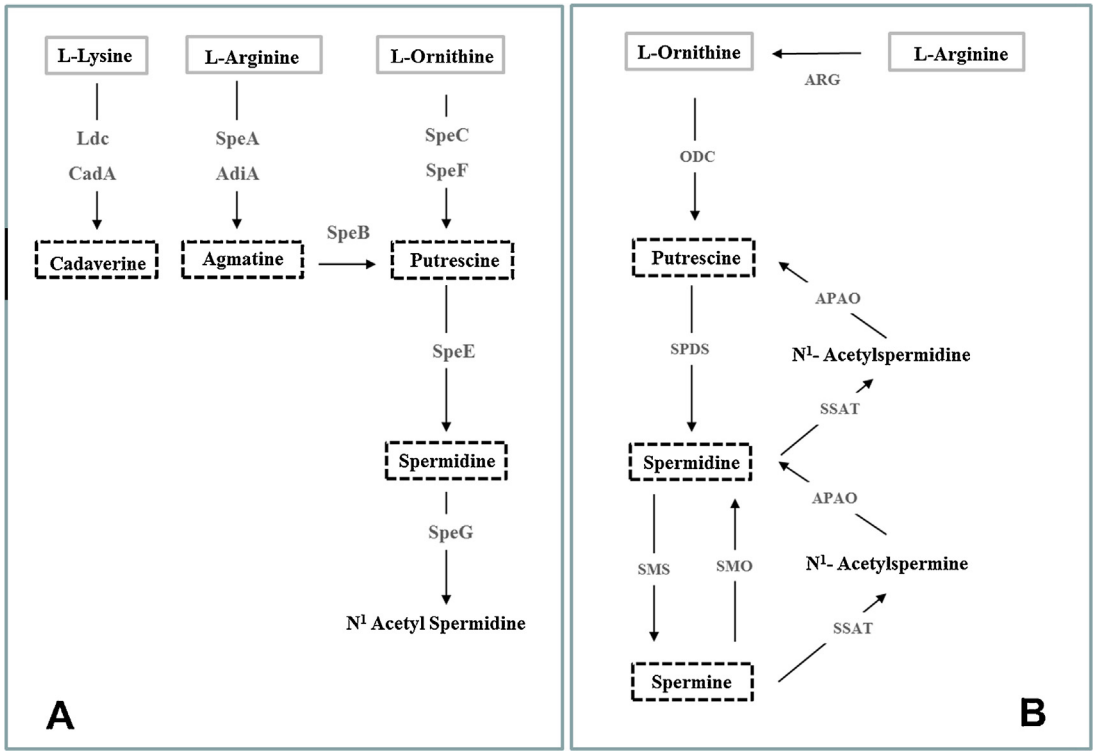

Fig. 2. Metabolism of the major polyamines. Schematic diagram depicting the pathway of polyamine biosynthesis in *E. coli* (A) and human cells (B) Most steps of the *E.coli* pathways exist also in other bacteria. *E. coli* possesses two forms of lysine, arginine and ornithine decarboxylase, a biosynthetic (or constitutive) form and a biodegradative (or inducible) form. Ldc, SpeA and SpeC are biosynthetic decarboxylases while CadA, AdiA and SpeF are the degradative ones. SpeE is the spermidine synthase and SpeG is the spermidine acetyltransferase (Keseler et al., 2011). ARG, Arginase; ODC, Ornithine decarboxylase; SPDS, Spermidine synthase; SMS, Spermine synthase; SMO, Spermine oxidase; SSAT, Spermine/spermidine acetyltransferase; APAO, Acetylpolyamine oxidase (Romero et al., 2004).

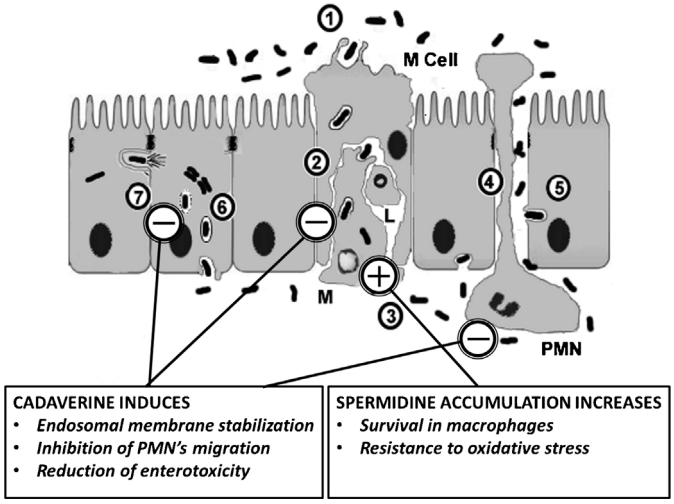

Fig. 3. Relevance of polyamines to the *Shigella* invasive process. *Shigella* infects the colonic epithelium *via* M cell translocation (1). After being endocytosed by M cells, the bacteria invade resident macrophages (2), where they destroy the endosomal membrane and survive within the cytoplasm. Bacterial multiplication within macrophages induces apoptosis and a massive inflammatory response (3). This results in transmigration of polymorphonucleated leukocytes (PMN) through the tight junctions between epithelial cells (4). *Shigella* released from dying macrophages enter the epithelial cells *via* the basolateral surface (5). After the invasion, the bacteria lose the vacuole membrane (6) and disseminate within the infected cells and the neighbouring ones (7). The contrasting effect of cadaverine and spermidine are reported in the boxes. M, Macrophage; L, Lymphocite; PMN, Polymorphonucleate leucocyte.

pathogenicity of *Shigella* stress the strong relevance of the intracellular concentration of these small molecules to the optimal fitness of the bacterium within the infected host.

Streptococcus: PotD, a polyamine transporter, is required for pathogenicity

Streptococcus pneumoniae is an encapsulated human pathogen that asymptomatically colonizes the nasopharynx. When it migrates from the upper respiratory tract and enters the lungs, it causes pneumonia. In some cases it can also cause bacteremia and meningitis. Pneumococcal infections are a serious public health concern because of their fatal outcome, especially in children, the elderly and immunocompromised patients (Kadioglu et al., 2008).

Pneumocci are able to synthesize spermidine and cadaverine using their spermine synthase (*speE*) and lysine decarboxylase (*cad*) activities. They also have a polyamine transport operon (*potABCD*) responsible for the binding and uptake of putrescine and spermidine. The first evidence of a potential role of polyamine-related genes in pneumocoocal pathogenesis comes from PotD, a surface-associated spermidine-/putrescine-binding protein highly homologous to the *E. coli* PotD (Polissi et al., 1998). In systemic and pulmonary murine models, silencing the *potD* gene of a virulent capsular *S. pneumoniae* strain significantly attenuates the progression of the disease (Ware et al., 2006) and immunization with PotD reduces mortality (Shah and Swiatlo, 2006). The involvement of polyamine transport systems in the survival strategies adopted by *S. pneumoniae* in the host is further stressed by the strongly enhanced expression of *potD* displayed during murine septicaemia and in response to stress conditions (*e.g.* temperature shock, oxidative stress, or choline limitation) that bacteria face within the host (Shah et al., 2008). Interestingly, an increase in extracellular choline results in a reduction of *potD* expression. Choline, a small molecule bridging pneumococcal theicoic acids with several proteins acting as virulence factors, is essential for pneumococcal virulence (Kharat and Tomasz, 2006). Because of the structural similarity between polyamines and choline, pneumococci can substitute choline with polyamines. In choline-restricted environments the increased expression of PotD may play a double role: PotD, besides binding and transporting the available choline into the cell, may also increase the intracellular pool of polyamines which then replace cholin during theicoic acid synthesis (Shah et al., 2008).

Besides *potD*, other polyamine-associated genes are involved in pneumococcal virulence. Recently Shah et al. (2011) have reported that strains deficient in polyamine biosynthesis or transport (*speE*, *cad* or *potABCD* mutants) are significantly attenuated in murine models of infections and showed reduced expression of several proteins involved in growth, replication and virulence. Even if the mechanisms responsible for the reduced fitness of the polyamine mutants within the host have not been elucidated, polyamines appear deeply connected to the survival and pathogenicity of *S. pneumoniae* and may represent appropriate targets for innovative therapeutic approaches.

Salmonella Typhimurium: polyamines promote the synthesis of Type III secretion systems

Salmonella enterica serovar Typhimurium (*S.* Typhimurium) is a facultative intracellular pathogen able to cause a wide variety of food- and waterborne diseases, ranging from self-limiting gastroenteritis to systemic and life-threatening infections. The ability of *S.* Typhimurium to cause disease is largely dependent on two Type III Secretion systems ($T3SS_1$ and $T3SS_2$), encoded by genes located within the *Salmonella* pathogenicity islands SPI_1 and SPI_2, respectively (Ibarra and Steele-Mortimer, 2009).

The SPI_1-encoded $T3SS_1$ injects a specific set of bacterial effector proteins into the epithelial cells lining the wall of the small intestines, thereby promoting the invasion of the host cells. SPI_2-located genes enable *S.* Typhimurium to survive and proliferate within specialized compartments inside the host cell. Indeed, within the so-called *Salmonella* containing vacuole (SCV), the bacterium uses $T3SS_2$ to inject a specific set of effectors across the vacuole membrane into the host cell cytosol, facilitating SCV maturation and migration towards the Golgi apparatus. Moreover, $T3SS_2$ and its secreted effectors also control intracellular replication. The expression of SPI_1 and SPI_2 genes is tightly controlled by multiple regulators organized in a complex network and induced by several environmental signals (Ibarra and Steele-Mortimer, 2009).

The function of polyamines in *S.* Typhimurium has remained largely unknown until the analysis of intracellular gene expression profiles has revealed that the expression of genes for the biosynthesis of spermidine and putrescine is up-regulated during the infection of epithelial cells and macrophages, thus suggesting a potential role in both invasion and intracellular survival (Eriksson et al., 2003; Hautefort et al., 2008). A comparative analysis of wt and *S.* Typhimurium strains defective in the biosynthesis of putrescine and spermidine (*spe*-mutant) has recently revealed that these polyamines play a critical role in virulence (Jelsback et al., 2012). Indeed, the *spe*-mutant displays defective invasion of the epithelial cells, shows reduced intracellular survival and is attenuated in a mouse model of typhoid fever. In contrast, an *S.* Typhimurium strain defective in the transport of putrescine and spermidine (*pot*-mutant) is not significantly affected in invasion (Jelsback et al., 2012). The reduced virulence potential of the *spe*-mutant is paralleled by the reduced expression of both, SPI_1 and SPI_2 genes. Moreover, transcriptional analysis reveals that in the SPI_1 locus of the *spe*-mutant, the master regulator *hilA* and the *inv* and *sip* operons are downregulated. Similarly, a significantly reduced expression is observed for several genes encoding $T3SS_2$ components and effectors. Interestingly, the exogenous presence

of putrescine and spermidine in the culture media prior to infection significantly enhances intracellular survival, suggesting that polyamines might function as an environmental signal that primes *S.* Typhimurium for invasion, multiplication and spread within the host.

Yersinia pestis: the crucial role of polyamines in the transmission from flea to mammal

Biofilm formation is an essential step in the pathogenicity process of *Yersinia pestis*, the etiological agent of bubonic plague (Hinnebusch and Erickson, 2008), a zoonotic disease transmitted to humans by flea bite. To produce a transmissible infection, *Y. pestis* grows as a biofilm attached to in the foregut of the flea host, inducing blockage of the valve which separates the oesophagus from the midgut. When the flea bites it regurgitates the bacterial biofilm into the dermis.

Biofilm formation in the flea normally occurs during bacterial growth at temperatures up to 34 °C and is controlled by the level and activities of six gene products of the hemin storage locus (*hms*) (Hinnebusch et al., 1996) grouped in three operons, *hmsHFRS*, *hmsT* and *hmsP*. Using *Y. pestis* mutants, defective in the synthesis of arginine decarboxylase (*speA*) or of ornithine decarboxylase (*speC*) (Fig. 2A), Patel et al. (2006) observed a drastic (95%) or partial (50%) depletion of putrescine. The complete loss of intracellular putrescine and spermidine, the two major polyamines present in *Y. pestis*, occurs only in the *speA speC* double mutant. Polyamine deficiency does not have an impact on cell growth but severely affects biofilm production. In particular, putrescine appears to be the major polyamine for biofilm production: in polyamine defective mutants, the addition of putrescine restores biofilm production in a dose dependent manner. Polyamines are necessary to maintain the level of three Hms proteins required for biofilm production: HmsR, HmsS and HmsT (Wortham et al., 2010). In particular, a reduced translation of the inner membrane protein HmsR and of HmsT, a diguanylate cyclase responsible for the formation of c-di-GMP, is the primary consequence of polyamine deprivation. Polyamines are known to promote translation irrespective of the presence of a canonical Shine Delgarno (SD) sequence (Yoshida et al., 2004) and, interestingly, *hmsR* and *hmsT*, the two genes most affected by polyamine loss, lack a consensus SD. Finally, in a mouse model of bubonic plague, a polyamine-defective mutant is significantly less virulent as compared to the wt. The same effect was not reproduced in a mouse model of pneumonic plague. This suggests that in the mammalian host, the availability of polyamines may be different during systemic or lung infections, or that polyamines may be required for the expression of other factors involved in the virulence of bubonic but not pneumonic plague (Wortham et al., 2010).

Staphylococcus aureus: from polyamine hypersensitivity to polyamine resistance

A fundamental biological property of *Staphylococus aureus* is the ability to asymptomatically colonize its host. The primary mode of transmission of *S. aureus* is by direct contact (usually skin-to-skin) with colonized or infected individuals. *S. aureus* infections are very difficult to treat, given the highly invasive nature of this microrganism combined with its multiple antimicrobial resistance determinants (Chambers and Deleo, 2009). Infections caused by antibiotic-resistant strains of *S. aureus*, in particular by methicillin-resistant strains (MRSA), have reached epidemic proportions all over the world. MRSA strains, historically associated with hospitals and other healthcare institutions, are a widespread cause of community infections. The so-called community-associated MRSA (CA-MSRA) strains spread rapidly among healthy individuals and are phylogenetically distinct from the traditional, hospital-associated (HA-MSRA) strains.

In recent years a clone belonging to the USA-300 group has been shown to be responsible for the vast majority of CA-MRSA diseases around the world (Diep et al., 2006). In order to explain the hypervirulence and hypertransmissibility associated with USA-300 clones, a comparative genome analysis of these clones and of the previously prevailing USA-400 CA-MRSA clones has been carried out (Diep et al., 2006). USA-300 clones harbour an Arginine Catabolic Mobile Element (ACME) which is rarely found in any other CA-MRSA or HA-MRSA clones (Diep et al., 2008). This 33 kb ACME island, besides containing several genes related to mobility, also harbours two genetic loci involved in polyamine metabolism, the *arc* system and the *speG* gene (Diep et al., 2008; Joshi et al., 2011). The *arc* system encodes a constitutive arginine deaminase which converts extracellular arginine to ornithine, a key intermediate in the synthesis of spermidine and spermine, while the *speG* gene encodes spermidine acetyltransferase (Fig. 2A).

Surprisingly, *S. aureus* lacks *de novo* polyamine biosynthetic genes and consequently does not produce detectable levels of polyamines. Exogenous polyamines inhibit *S. aureus* growth rather than enhancing it, and can be bactericidal at concentrations known to exist within the human host (Joshi et al., 2011). In contrast to the unique polyamine hypersensitivity displayed by all *S. aureus* strains, USA-300 clones exhibit innate resistance to spermine and spermidine (Joshi et al., 2011). Recently it has been demonstrated (Thurlow et al., 2013) that the ACME element directly contributes to the persistence of USA-300 clones within cutaneous abscesses and in environments that mimic human skin. In particular, the ACME *arc* system allows USA-300 to thrive in the acid skin environment. However once US-300 penetrates the epidermis, excess ornithine generated by the *arc* system drives an excessive synthesis of polyamines in the host, thereby necessitating SpeG-mediated polyamine detoxification (Thurlow et al., 2013).

The crucial role played by ACME *speG* is clearly demonstrated by the observation that USA-300 *speG* mutants recover the polyamine hypersensitive phenotype and that the introduction of a functional *speG* gene in non-USA-300 clones confers polyamine resistance (Joshi et al., 2011). Moreover, the inhibition of the *de novo* synthesis pathway in the host restores the persistence of a US-300 *speG*-defective strain within infected wounds but also severely affects the host healing process, suggesting that polyamines play an essential role in coordinating the wound-healing response of the host to *S. aureus* infections.

Vibrio cholerae: the involvement of polyamines in biofilm formation

Vibrio cholerae, the causative agent of cholera, a devastating diarrheal disease, is a natural inhabitant of aquatic environments where it is believed to exist predominantly as biofilms on the outer surfaces of many organisms, such as crustaceans, zooplankton and plants. Biofilms contribute to bacterial persistence in the human host as well. Indeed, they have been correlated with enhanced infectivity of *V. cholerae* because of their capacity to carry a high dose of the pathogen and to provide protection against host natural defence mechanisms (Faruque et al., 2006). Biofilm development is a complex phenomenon, controlled by a variety of environmental signals, such as osmolarity and quorum signals, and by the presence of small molecules like nucleosides, bile salts and polyamines.

The dominant polyamine in *Vibrio* spp is norspermidine, a structural analogue of spermidine (Fig. 1), acting also as a backbone

scaffold in the synthesis of the siderophore vibriobactin (Griffiths et al., 1984). Norspermidine is synthetized by an alternative polyamine biosynthetic pathway, based on aspartate β-semialdehyde as aminopropyl group donor and on the activity of two enzymes, carboxynorspermidine synthase (CANSDH) and carboxynorspermidine decarboxylase (CANSDC). By using CANSDH- or CANSDC-defective *V. cholerae* strains, norspermidine has been demonstrated to be essential for biofilm formation. Deletion of either the CANSDH- or the CANSDC- enconding gene severely affects the growth of *V. cholerae* planktonic cells, as well as the production of biofilm (Lee et al., 2009). The increased expression of the CANSDH gene stimulates the *vps* gene, which is involved in the synthesis and export of the biofilm exopolysaccharide matrix (Parker et al., 2012). When norspermidine is supplied extracellularly, it is able to enhance biofilm formation by a different mechanism involving the norspermidine sensor protein NspS (Karatan et al., 2005): the binding of norspermidine to NspS might modify the interaction of NspS with the transmembrane protein MbaA and determine the activation of biofilm-associated genes. These acquisitions notwithstanding, depletion of norspermidine does not attenuate *V. cholerae* infectivity in an infant mouse model of infection. This may be justified by hypothesizing that in the gut a significant amount of norspermidine exists, ready to be taken up by the infecting bacteria.

Recently, the analysis of *V. cholerae* mutants defective in the PotD1 spermidine transporter (McGinnis et al., 2009) revealed a significant increase in biofilm formation, suggesting that spermidine might negatively interfere with biofilm production. This is further confirmed by the observation from the same authors that the supply of exogenous spermidine hampers biofilm synthesis. A plausible possibility is that spermidine competes with norspermidine for the binding to the NspS sensor, thus hindering the formation of the NspS/MbaA complex required for the activation of biofilm genes (McGinnis et al., 2009). While the molecular mechanisms underlying this competition are as yet not fully elucidated, the observations available so far suggest that in *V. cholerae* different polyamines interfere with biofilm formation by targeting different regulatory pathways.

Pseudomonas aeruginosa: a spermidine trasporter as signal modulator of a Type III secretion system

Pseudomonas aeruginosa is an important opportunistic pathogen that causes serious infections in immunocompromised individuals (Driscoll et al., 2007). The ability of *P. aeruginosa* to avoid phagocytic clearance is a major virulence determinant: successful evasion from phagocytosis depends primarily on the presence of a functional type III secretion system (T3SS), able to traslocate four effectors (ExoS, -T, - U, and -Y) into the host cells. In particular, while ExoU and ExoY are cytotoxins with phospholipase and adenylate cyclase activities, ExoS and ExoT are involved in the inhibition of phagocytosis by disrupting actin cytoskeletal rearrangements and by negatively interfering with signal transduction cascades. All T3SS genes are under the control of ExsA (an AraC like activator which binds a specific sequence motif of the T3SS promoters) and are strongly induced by host cell contact and low calcium concentration (Yahr and Wolfang, 2006). Silencing of the major spermidine uptake system, encoded by the *spu*EFGH genes (Lu et al., 2002), decreases the expression of most T3SS genes, including the master regulator operon *exsCEBA* (Zhou et al., 2007). In particular, deletion of the *spuE* gene disables the low calcium-induced expression of the *exsCEBA* operon, while exogenous addition of spermidine increases its expression in a dose-dependent manner. Interestingly, spermidine alleviates the repression of calcium on T3SS gene expression (Zhou et al., 2007). The recent definition of the crystal structure of SpuD and SpuE (Wu et al., 2012) reveals that SpuD preferentially binds putrescine over spermidine, whereas SpuE is a spermidine-specific binding protein. As opposed to SpuD, the inactivation of SpuE decreases the host cell contact induction of the T3SS system and attenuates bacterial citotoxicity of *P. aeruginosa*, suggesting that the bacterium may exploit the spermidine uptake system as a novel T3SS activator (Wu et al., 2012). The discovery that spermidine can activate a T3SS in the presence of calcium concentrations as high as those found in the human body, provides an interesting model of functional co-evolution of the T3SS regulatory pathway and of the spermidine transporter-mediated signalling system.

Francisella tularensis: polyamines limit the proinflammatory host response

Francisella tularensis, the causative agent of tularemia, is a highly virulent facultative intracellular pathogen which has raised considerable interest in the past few years as potential agent of biowarfare. *F. tularensis* is highly infectious and causes a fatal febrile syndrome (tularaemia) in humans (McLendon et al., 2006). Macrophages and dendritic cells are rapidly infected by *F. tularensis*, becoming a relevant replicative niche for the bacterium. Within these cells, *F. tularensis* actively inhibits intracellular signalling, including NF-kB activation, thereby limiting the proinflammatory host response.

By analysing cytokine production in human macrophages it has been shown that the stimulation of TNF-α and other proinflammatory cytokines by *F. tularensis* is reduced when bacteria are grown in the presence of spermine or spermidine prior to infection (Carlson et al. 2009). The relevance of the polyamines within host cells infected by *Francisella* has been confirmed by the observation that a large portion of the *Francisella* transcriptome changes in response to spermine. On the basis of these results Carlson et al. (2009) proposed a model in which spermine or spermidine found in the cytosol of macrophages elicit substantial changes in *Francisella* gene expression, culminating in the disruption of innate immune cell functions.

Recently in the live vaccine strain (LVS) of *F. tularensis*, transposon mutagenesis has allowed to identify a genetic locus (FTL 0883) required to minimize the activation of macrophages (Russo et al., 2011). A homologous gene (FTT 0615c) has been found in the *F. tularensis* virulent strain Schu S4. Strains defective in these loci induce strong activation of murine and human macrophage response and are therefore significantly attenuated *in vivo*. The function of the proteins encoded by FTL 0883 and FTT 0615c is as yet undefined, but it has been hypothesized (Russo et al., 2011) that these proteins, predicted to be located in the inner membrane, may somehow sense the host polyamine level and directly or indirectly modulate the spermine response of *Francisella*.

Helicobacter pylori: an astute exploitation of host cell polyamines

Helicobacter pylori is a microaerophilic bacterium that selectively colonizes the mammalian stomach and causes gastritis, peptic ulcers and gastric cancers. Interestingly, there is a strong innate and adaptive immune response by the host, but the outcome is lifelong gastritis without eradication of the organism. *H. pylori* is able to enhance its own survival in the face of the immune response by an ingenious manipulation of the polyamine metabolism of the targeted host cells (Chaturvedi et al., 2004, 2010, 2011).

One potential cause of ineffective immune response against *H. pylori* is the induction of apoptosis in macrophages due to polyamine oxidation. Macrophages respond to *H. pylori* by activating arginase II (Arg2) and ornithine decarboxylase (ODC), both

Table 1
Major contributions of polyamines in bacterial pathogenesis.

Bacterial pathogen	Polyamine-related strategy	Major effect	Reference
Shigella spp	Intracellular accumulation of spermidine	Higher resistance to oxidative stress, increased survival in macrophages	Barbagallo et al. (2011)
	Elimination of cadaverine	Optimization of the proinflammatory response and of the invasive process	Maurelli et al. (1998) and Bliven and Maurelli (2012)
Streptococcus penumoniae	Increased *in vivo* expression of PotD transporter	Expression of virulence factors and of genes involved in host colonization	Ware et al. (2006)
Salmonella Typhimurium	Increased *in vivo* biosynthesis of putrescine and spermidine	Activation of the SPI_1- and SPI_2-encoded virulence genes, including $T3SS_2$	Jelsback et al. (2012)
Yersinia pestis	Synthesis of bacterial putrescine and spermidine	Translation of genes involved in biofilm formation	Patel et al. (2006) and Wortham et al. (2010)
Staphylococcus aureus USA-400 CA-MRSA	ACME-dependent synthesis of spermidine acetyltransferase	Loss of polyamine hypersensitivity	Joshi et al. (2011)
Vibrio cholerae	Synthesis of bacterial norspermidine	Expression of genes involved in biofilm formation	Lee et al. (2009)
Pseudomonas aeruginosa	Activation of the Spu spermidine uptake system	Expression of the *exsCEBA* master regulator of T3SS in the presence of calcium	Zhou et al. (2007)
Francisella tularensis	Exploitation of host spermine and spermidine	Disruption of the innate immunte cell function	Carlson et al. (2009)
Helicobacter pylori	Induction of host polyamine oxidation	Macrophage apoptosis and dysregulation of the innate immune response.	Chaturvedi et al. (2004)
	Induction of the host Arg/ODC pathway	Weakening of the host defense by suppression of NO production	Chaturvedi et al. (2010)
	GacA-mediated induction of polyamine oxidation	DNA damage and gastric carcinogenesis	Chaturvedi et al. (2011)
Legionella pneumophila	Exploitation of host polyamines	Optimization of intracellular growth	Nasrallah et al. (2011)
Burkolderia pseudomallei	Synthesis of bacterial spermidine	Induction of genes involved in *quorum sensing* and biofilm formation	Chan and Chua (2010)

Abbreviations: SPI, Salmonella Pathogenity Island; T3SS, Type III Secretion System; ACME, Arginine Catabolic Mobile Element; NO, nitric oxide; ODC, ornithine decarboxylase; CA-MRSA, Community Acquired Multiresistant *S. aureus*.

involved in polyamine synthesis, and spermine oxidase, (SMO), responsible for the catabolic oxidation of spermine (Fig. 2B). In particular, following *H. pylori* infection L-arginine is transported into macrophages and metabolized by Arg2 into L-ornithine which ODC then converts into putrescine, the precursor of spermidine and spermine. The increased level of polyamines determines macrophage apoptosis, since spermidine and spermine are oxidized by the *H. pylori*-induced SMO causing the release of hydrogen peroxide and the depolarization of the mitochondrial membrane (Chaturvedi et al., 2004). These processes only affect polyamines synthesized by macrophages in response to the infection: no detectable polyamine levels have been found in *H. pylori* bacterial cultures used for the macrophages stimulation.

The ineffectiveness of the innate immune response in eradicating *H. pylori* infections is also due to a severely reduced production of nitric oxide (NO), a fundamental player in innate immunity (Chaturvedi et al., 2010). The *H. pylori*-mediated induction of the Arg2/ODC pathway negatively interferes with the translation of the inducible nitric oxidase synthase (iNOS), thus reducing NO production. The inhibition of NO synthesis leads to a decreased killing of *H. pylori* in its gastric niche. Among polyamines, spermine plays is crucial in this process: an exogenous supply of this polyamine increases *H. pylori* survival in a concentration-dependent manner.

Besides determining dysregulation of the innate immune response, during *H. pylori* infections polyamines induce oxidative damage in gastric epithelial cells. As in macrophages, also in this case the *H. pylori*-induced SMO activation promotes polyamine oxidation with the consequent production of reactive oxygen species (ROS) causing both, apoptosis and DNA damage (Xu et al., 2004). The induction of SMO in gastric epithelial cells relies on a major *H. pylori* virulence factor, the CagA cytotoxin (Chaturvedi et al., 2011). Following infection, *H. pylori* also activates the epithelial growth factor receptor (EGFR). This attenuates gastric epithelial cell apoptosis and favours the survival of cells with damaged DNA (Yan et al., 2009). The persistence of DNA damage is commonly recognized as a critical step in the development of neoplastic transformations. In this context it is worth mentioning that chronic gastritis induced by *H. pylori* is the strongest known risk factor for gastric adenocarcinoma (Chaturvedi et al., 2011), a leading cause of cancer-related death.

Conclusions

During recent years, an increasing number of studies on the function of polyamines in bacteria have brought new insight in the active role of these molecules during different steps of the pathogenic processes of different virulent species. In this review, we have summarized major acquisitions (Table 1) showing how relevant human pathogens have developed their own resourceful ways to exploit, manipulate or interfere with polyamines and polyamine-related processes to optimize their fitness within the host. Supplementary evidence exists stressing the relevance of polyamines to bacterial pathogenicity: *Legionella pneumophila* has been recently shown to effectively exploit host polyamines for the optimization of its intracellular growth (Nasrallah et al., 2011), while bacterial polyamines contribute to the induction of quorum sensing in *Burkolderia pseudomallei* (Chan and Chua, 2010).

Overall, the interplay among polyamines, host cell metabolism and bacterial pathogenesis is complex and strongly diversified. However, some common traits in the strategies adopted might be envisaged. In *Shigella* spp.and *L. pneumophila*, polyamines favour bacterial survival within the host, though *Shigella* banks on its own polyamine pool (Prosseda et al., 2012) whereas *L. pneumophila* depends on host polyamines (Nasrallah et al., 2011). Other pathogens require polyamines to promote the expression of virulence determinants following infection of the host. This happens typically in *S.* Typhimurium and *P. aeruginosa* where polyamines are necessary for the full induction of T3SS systems (Jelsback et al., 2012; Zhou et al., 2007), and in *S. pneumoniae*, which requires a polyamine transporter for the full expression of the virulence phenotype (Shah et al., 2008). An ingenious variant of this strategy is found in *Y. pestis*, *V. cholerae* and *B. pseudomallei*, where polyamines are crucial for triggering the genes involved in biofilm formation (Chan and Chua, 2010; Lee et al., 2009; Wortham et al., 2010) Another strategy is best exemplified by *H. pylori*, where

the oxidation of host polyamines is deeply linked to the dysregulation of the innate immune response and to the induction of DNA damage (Chaturvedi et al., 2004, 2011). Preliminary evidence indicates that also *F. tularensis* exploits polyamines to alter the host immune response (Russo et al., 2011). Finally, there is the case of *S. aureus*, where measures defeating hypersensitivity to polyamines result in increased virulence (Joshi et al., 2011).

Considering the capability of bacteria to rapidly adapt to new environments, further investigations are expected to shed more light on additional bacterial strategies to turn polyamine metabolism to their own advantage. Besides contributing to a better understanding of the intricate relationship between a pathogen and its host, further investigations on the role of polyamines may open new avenues for the development of novel antibacterial strategies based on inhibitors of polyamine synthesis and transport systems.

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