Chapter 5

Calcium Signaling in Prokaryotes

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Abstract

Calcium (Ca^{2+}) functions as a universal messenger in eukaryotes and regulates many intracellular processes such as cell division and gene expression. However, the physiological role of Ca^{2+} in prokaryotic cells remains unclear. Indirect evidence suggests that Ca^{2+} is involved in a wide variety of bacterial cellular processes including membrane transport mechanisms (channels, primary and secondary transporters), chemotaxis, cell division and cell differentiation processes such as sporulation and heterocyst formation. In addition, Ca^{2+} signaling has been implicated in various stages of bacterial infections and host-pathogen interactions. The most significant discovery is that similar to eukaryotic cells, bacteria always maintain very low cytosolic free Ca^{2+}, even in the presence of millimolar extracellular Ca^{2+}. Furthermore, Ca^{2+} transients are produced in response to stimuli by several agents. Transport systems, which may be involved in Ca^{2+} homeostasis are present in bacteria but none of these have been examined critically. Ca^{2+}-binding proteins have also been identified, including proteins with EF motifs but their role as intracellular Ca^{2+} targets is elusive. Genomic studies indicate that changes in intracellular Ca^{2+} up and downregulate hundreds of genes and proteins suggesting a physiological role. This chapter presents an overview of the role of Ca^{2+} in prokaryotes summarizing recent developments.

Keywords: Ca^{2+} signaling in bacteria, calcium binding proteins, Ca^{2+} homeostasis in bacteria, prokaryotic Ca^{2+} transporters

1. Introduction

Intracellular free Ca^{2+} serves as a universal messenger in all eukaryotic cells [1–4]. Cells respond to environmental stimuli by transient changes in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), which are utilized by cells to transmit information. Physiological responses also depend on the speed, magnitude and spatiotemporal patterns of the Ca^{2+} signal [5]. Basal

levels of free cytosolic calcium are regulated by Ca^{2+}-binding proteins, primary and secondary transporters and cytosolic Ca^{2+} stores preventing calcium phosphate toxicity [1, 3].

Although the role of Ca^{2+} in prokaryotes is still unclear, there is increased evidence favoring a role for ($[Ca^{2+}]_i$) in signal transduction in bacteria. Indirect evidence shows that Ca^{2+} affects several bacterial physiological processes including: chemotaxis, cell differentiation such as spore development and heterocyst formation, membrane transport (channels, primary and secondary transporters), virulence and host pathogen interactions [4, 6–10]. Similar to eukaryotes, bacteria maintain cytosolic free Ca^{2+} within the nM range even in the presence of mM extracellular Ca^{2+} [11–15]. Ca^{2+}-stimulus-response has been documented during environmental stress, toxicants [16–18] carbohydrate metabolites [19, 20], iron acquisition, quinolone signaling and type III secretion, which are secretory systems comprised of proteins found in pathogenic Gram negative bacteria that are used to infect eukaryotic cells [21, 22]), suggesting that Ca^{2+} signals are relevant to microbial physiology. Primary and secondary transporters including channels (Ca^{2+}, K^+, Na^+) have been identified in various genera of bacteria. Data show that the level of similarity with eukaryotic counterparts is striking. For example sodium channels show high degree of conservation but their structure is simpler [23]. The ATPase found in *B. subtilis* is analogous to the typical eukaryotic type IIA family of P-type ion-motive ATPases [24]. However, direct evidence that these transporters regulate the concentration of cytosolic free Ca^{2+} is limited. There is evidence of calcium binding proteins (CaBP) in several genera of bacteria, including proteins with EF-hand domains [25, 26], and other calcium motifs such as β-rolls motif, Greek key motif, repeats in toxin and Big Ca^{2+} domain [27–30] but their functional role needs to be investigated. Proteomic and transcriptomic studies in *E. coli*,

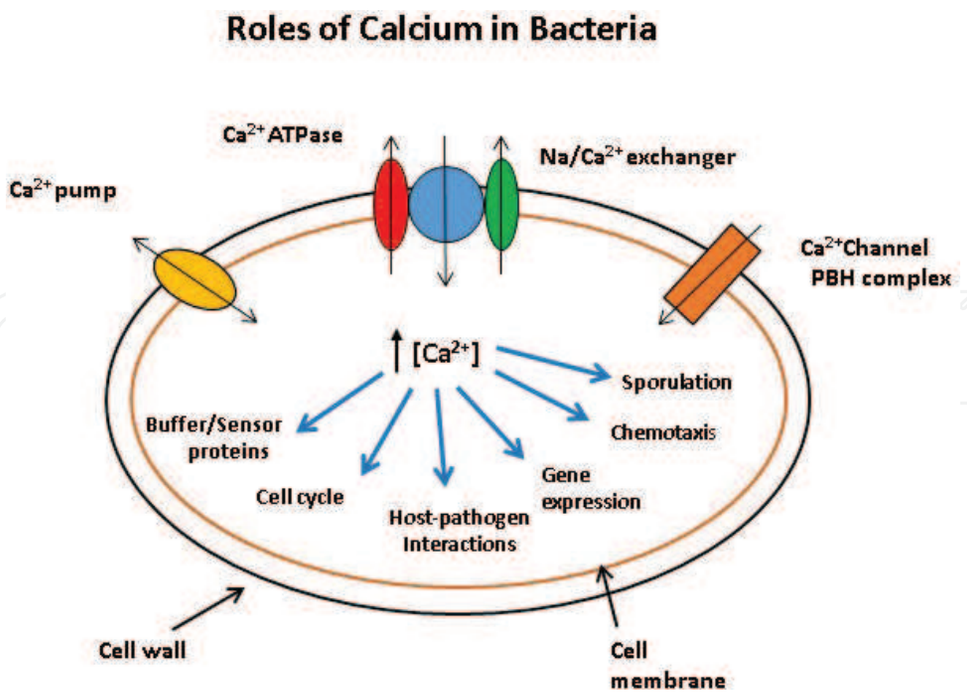

Figure 1. Possible roles of calcium in bacteria.

P. aeruginosa and *B. subtilis* showed that hundreds of genes and proteins are up/downregulated by changes in ($[Ca^{2+}]_i$) but the physiological role needs to be elucidated [20, 21, 23, 31, 32]. **Figure 1** illustrates the potential roles of Ca^{2+} in prokaryotes.

Despite the progress made in recent years, the role of Ca^{2+} in prokaryotes remains intriguing and unclear. Disappointingly many studies have not been followed up and the understanding of the role of Ca^{2+} in prokaryotes lags behind. Questions that need to be answered are: why bacteria maintain a very low cytosolic free Ca^{2+}? Do bacteria utilize the high Ca^{2+} gradient to trigger cell events? What are the molecular mechanisms for Ca^{2+} regulation in bacteria? Does intracellular Ca^{2+} play a role in provoking and regulating cell events? This chapter reviews the work done in this field and will present recent developments.

2. Ca^{2+} homeostasis in bacteria

Initial measurements of $[Ca^{2+}]_i$ in bacteria were a challenge because of the unique physical characteristics of bacterial cells (tiny size, cell walls and membrane), the difficulty in manipulating live cells and the toxicity of reagents [13, 33]. Other concerns included those associated with Ca^{2+} research such as contamination and lack of selectivity of Ca^{2+} chelators [34–36]. With the introduction of molecular technology, the photoprotein aequorin gene was expressed in bacterial cells to measure cytosolic free Ca^{2+} in live cells. In this way, several investigators were able to continuously monitor cytosolic free-Ca^{2+} in several genera of bacteria [12–14]. A crucial discovery was that all bacteria tested maintained very low levels of cytosolic free Ca^{2+}, even in the presence of 1–10 mM extracellular Ca^{2+} (**Figure 2**). Cytosolic free Ca^{2+} in bacterial cells ranges from 100 to 300 nM, very similar values to those observed in eukaryotic cells [11, 13, 14]. These findings suggest that microbial cells must have transport systems (influx and efflux), proteins or other structures that may serve as intracellular free Ca^{2+} targets that may play a role in the maintenance of Ca^{2+} homeostasis.

The role of channels, ATPases and exchangers in Ca^{2+} homeostasis has not been investigated critically and none of these have been experimentally proven to transport Ca^{2+} specifically. The contribution of bacterial CaBP to Ca^{2+} homeostasis remains undetermined [26, 37]. However, recent work shows that the disruption of particular ATPases (PA2435, PA3920), the exchanger (PA2092) and a putative EF-hand protein, is evidence that these transporters are necessary to maintain low intracellular Ca^{2+} levels in *P. aeruginosa* [15, 38]. A proteomic analysis in *B. subtilis* showed that several cytosolic proteins appear to bind Ca^{2+}, as determined by Ca^{2+} autoradiography [32]. Some of these proteins, identified by liquid chromatography/mass spectrometry include: a potential cation transport ATPase, fructose biposhate aldolase, DnaK 70 and adenylate kinase. These proteins were induced when cells were treated with extracellular divalent cation chelator ethylene glycol tetraacetic acid (EGTA) and reduced when treated with high extracellular Ca^{2+}. None of these proteins however had Ca^{2+} binding domains [32]. Notably genes encoding fructose biposhate aldolase, DnaK 70 and adenylate

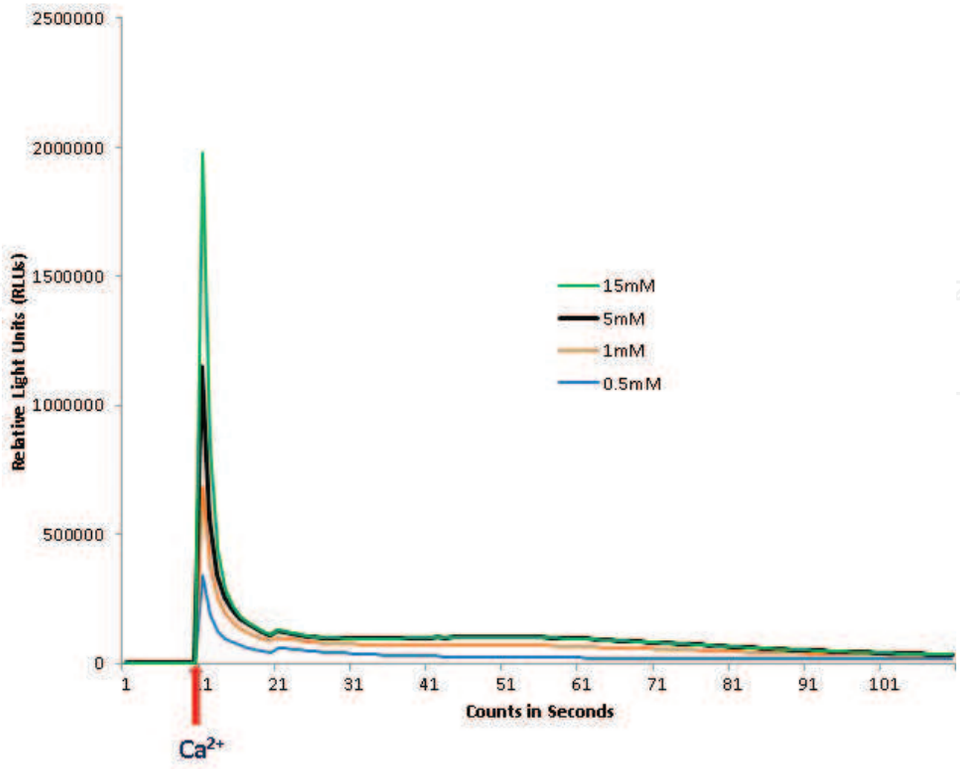

Figure 2. Cytosolic free Ca^{2+} in Bacillus subtilis cells. *B. subtilis* Cells were transformed with a plasmid containing the gene for the photoprotein aequorin. Light emission was recorded in a luminometer after challenging the cells with different $CaCl_2$ concentrations: 0.5, 1, 5, and 15 mM. J Anal Bioanal Tech reproduced with permission.

kinase were found to be modulated by Ca^{2+} in *E. coli* [20]. These findings suggest that perhaps other proteins and anionic protein groups yet to be characterized may be involved in buffering intracellular free Ca^{2+}.

Bacterial cells lack organelles such as endoplasmic reticulum and mitochondria, which function as Ca^{2+} sinks in eukaryotes. However, some bacteria contain membrane-bound vesicles (acidocalcisomes) and polyphosphate granules that accumulate and store Ca^{2+} [39–42]. Other structures that bind Ca^{2+} in significant amounts are DNA and the complex poly-(R)-3 hydroxybutyrate (PHB)-polyphosphate (PP) [43–45]. Moreover, the periplasmic space, which is a region between the inner cytoplasmic membrane and the bacterial outer membrane and that has been found in both Gram negative and Gram positive bacteria [46–48], is another structure that has been reported that may play a role in storing and buffering Ca^{2+} [49]. Intracellular free Ca^{2+} measurements within the periplasmic space in live *E. coli* cells revealed that this structure can store 3–6-fold Ca^{2+} with respect to the external medium [49]. Chang and co-workers [50] also demonstrated high concentrations of Ca^{2+} associated with the cellular envelope in *E. coli* cells as determined by X-ray mapping and electron loss spectroscopy.

Altogether, the aforementioned data suggest that bacterial cells may have different mechanisms to maintain cytosolic Ca^{2+} homeostasis. Further work should be performed to elucidate how and why bacterial cells maintain low levels of intracellular free Ca^{2+}.

3. Influx and efflux transport systems in bacteria

3.1. Influx

The existence of cation (Na^+ and K^+) and anion (Cl^-) channels, ATPases and exchangers have been documented in several genera of bacteria [4, 51]. Despite high resolution structure of some bacterial channels the physiological function reminds unknown [7]. Several bacteria have mechanosensitive ion channels that have large conductances (nanosiemens range) thus it would be expected to allow Ca^{2+} into cells. However, gene knockouts of major mechanosensitive channels in *E. coli* (MscL and MscS) still showed large Ca^{2+} influx [2, 52] and the Ca^{2+}-dependent K^+ channels of the archaea *Methanobacterium thermoautotrophicum* and *Thermoplasma volcanium* are activated at millimolar Ca^{2+} concentrations questioning the physiological relevance since Ca^{2+} signals occur within micromolar range. On the other hand, deletion of the SynCaK, a Ca^{2+}-dependent K^+ channel in cyanobacteria resulted in increased resistance to heavy metals suggesting a physiological role for Ca^{2+}-mediated channels [53].

So far the best evidence of a Ca^{2+} influx channel in bacteria is the nonproteinaceous complex polyhydroxybutyrate-polyphosphate (PHB-PP). The channel is highly selective for Ca^{2+} at a physiological pH [54]. This preference has been attributed to a high density negative charge along the polyphosphate backbone. The complexes are abundant in stationary phase and correlate with high rise in cytosolic Ca^{2+}. These complexes have many characteristics of protein Ca^{2+} channels: voltage-activated, conduct Ca^{2+}, Sr^{2+} and Ba^{2+} and are blocked in a concentration-dependent manner by La^{3+}, Co^{2+} and Cd^{2+} [44, 45, 55]. However, the genes encoding the synthesis of PHB complex remain to be properly identified and characterized. A figure of the putative channel is shown in **Figure 3**.

More recently, Bruni et al. [52] employing a sensor that simultaneously reports voltage and Ca^{2+} showed that Ca^{2+} influx is induced by voltage depolarization in *E. coli*. These exciting findings support the idea that bacteria may sense their environment through voltage-induced Ca^{2+} fluxes, similar to eukaryotic cells.

3.2. Efflux

In most bacteria, Ca^{2+} is apparently exported by Ca^{2+} exchangers, Ca^{2+}/H^+ or Ca^{2+}/Na^+ antiporters. These are low-affinity Ca^{2+} transport systems that use the energy stored in the electrochemical gradient of ions. Ca^{2+} exchangers differ in ion specificity and have been identified in a number of bacterial genera [11, 56]. In *E. coli*, the proteins ChaA, YrbG and PitB were reported as potential Ca^{2+}/H^+ [57, 58], Ca^{2+}/Na^+ antiporters [59] and Ca^{2+}/PO_4^{3+} symporter respectively. Knockout of corresponding genes showed no effect on either Ca^{2+} influx or efflux [19, 20] raising questions about the role of these proteins. Potential redundancy is not ruled out. More recently, the multidrug transporter LmrP from *Bacillus lactis* has a predicted EF-hand motif with a Kd = 7.2 μM and two acidic residues (Asp-235 and Glu-327) binding Ca^{2+}. LmrP was shown to selectively bind Ca^{2+} and Ba^{2+} and mediates selective Ca^{2+} efflux via electrogenic exchange [60]. A predicted transporter PA2092 from *P. aeruginosa* might be involved in Ca^{2+} efflux since intracellular Ca^{2+} accumulates after disruption of the corresponding mutant [15].

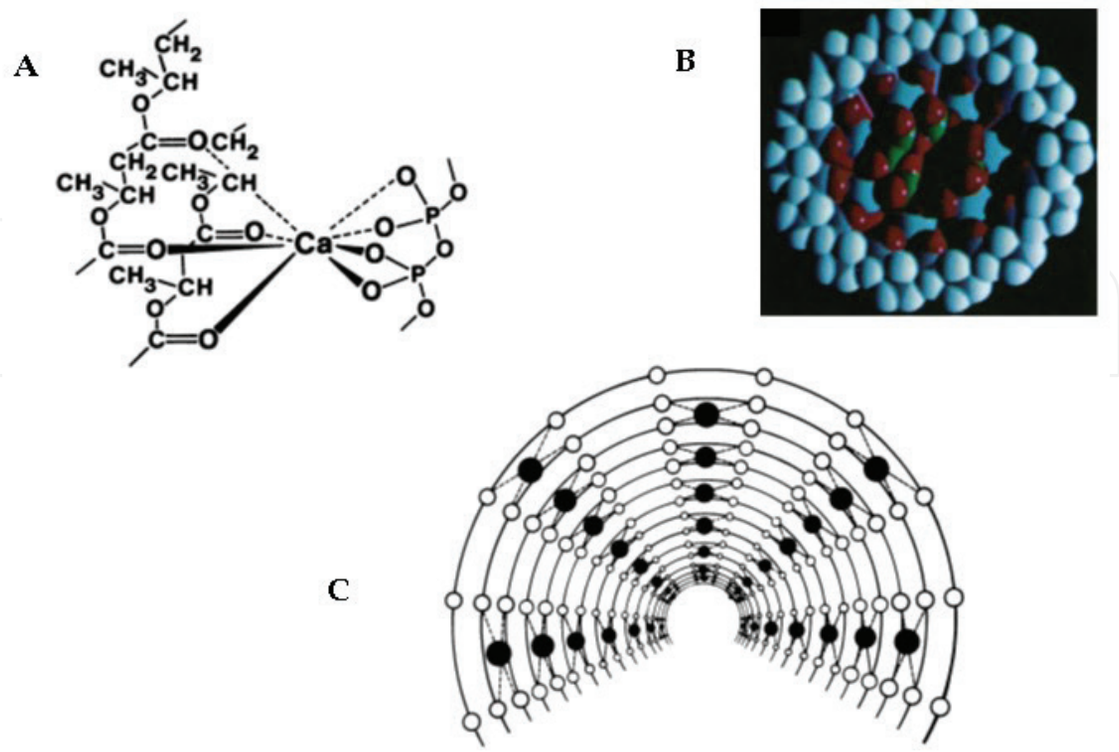

Figure 3. Coordination geometry of Ca^{2+} in the PHB-PP helix. (A) Calcium forms ionic bonds with four phosphoryl oxygens of poly-P and ion-dipole bonds with four ester carbonyl oxygens of poly-hydroxybutyrate (PHB) to form a neutral complex with distorted cubic geometry. (B) Computer model horizontal cross section showing the poly-P helix with the poly (HE) helix with Ca^{2+} surrounded by the oxygen moieties of both polymers. The seven Ca^{2+} displayed are from two turns of the poly-P helix. Light blue, hydrogen; dark blue, carbon; red, oxygen; green, phosphorous; aqua, Ca^{2+}. (C) View down the poly(HB) cylinder. Ca^{2+} (closed circles) bound to carbonyl ester oxygens (open circles) in a pattern that links each turn of the helix alternatively to the proximal turns above and below. Reusch and Sadoff [45]. Courtesy of Reusch RN.

P and F-type Ca^{2+} ATPases have been described in bacteria. ATPases that were purified and shown to translocate or have Ca^{2+}-dependent phosphorylation include:

the P-type ATPase from *Synechocystis sp.* showed vanadate sensitivity, which appears to be homologous to eukaryotic SERCA [61, 62], the F-type ATPase from *Flavobacterium odoratum* also vanadate-sensitive, phosphorylated only in the presence of Ca^{2+} [63] and the *Listeria monocytogenes* ATPase, which has low Ca^{2+} affinity, and it is induced at alkaline pH [64]. The *in vivo* function of these proteins remains to be characterized. Other ATPases that have been identified by bioinformatics include: CaxP from *Streptococcus pneumoniae* [65], YloB from *B. subtilis* [24], PacL from *Synechococcus* sp. [66] and PA2435 and PA3920 from *P. aeruginosa* [15].

Work by Naseem et al. [20] demonstrated that ATP is essential for Ca^{2+} efflux, and there is a possibility that ATP may regulate Ca^{2+} efflux through an ATPase. It was shown that the gene atpD, which encodes a component of an F-type ATPase is required for a normal Ca^{2+} efflux function. Although no specific transporter was shown here, the result is important, indicating that ATP is surely necessary for transport of Ca^{2+} by a still unknown ATPase.

Bacterial transporters have not been studied systematically and knowledge about these proteins is limited. It appears that prokaryotes have multiple transporters with some redundancy.

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Besides protecting from toxic effects the question arises is Ca^{2+} transport in bacteria linked to signaling? What is the contribution of these transport systems in Ca^{2+} homeostasis?

4. Bacterial Ca^{2+} binding proteins (CaBP)

If a change in cytosolic free Ca^{2+} is to have any effect on bacterial physiology, bacterial cells must have intracellular Ca^{2+} targets in addition to influx and efflux mechanisms. Identification of such intracellular Ca^{2+} targets remains elusive. Nevertheless, a number of prokaryotic CaBP have been discovered by a combination of approaches: molecular technology and bioinformatics. According to Zhou et al. [26], sequence analyses of prokaryotic genomes showed the presence of 397 putative EF-hand proteins. However, most of these proteins with a few exceptions (Calerythrin from *Saccharopolyspora erythrea*, Calsymin from *Rhizobium etli*, the *Brucella abortus* Asp24, *Streptomyces coelicolor* CabA, CabD and Ccbp from *Anabaena* sp.) are hypothetical proteins [37, 67, 68]. Few proteins have been studied biochemically and none of these have been characterized functionally.

Five classes of EF-hand motifs have been reported in bacteria. The typical helix-loop helix EF-hand structure seen in Calerythrin and Calsymin, the *ex*tracellular *Ca*$^{2+}$-*b*inding *r*egion (Excalibur), which has a shorter loop containing 10 residue motif DxDxDGxxCE found in various bacteria, the longer 15 residue Ca^{2+}-binding loop seen in the *E. coli* lytic transglycosylase B, and the fourth and fifth classes lacking the first or second helix as described in the *C. thermocellum* dockerin and the *Sphingomona* ssp. alginate-binding protein, respectively [25, 26]. **Table 1** presents the five classes of bacterial EF-hand and EF-hand-like motifs proteins with known structures. The presence of the Ca^{2+} binding motifs must be tested for functional necessity or for viability of the organism.

Other Ca^{2+} motifs found in various bacteria include the Ca^{2+}-binding β-roll motif, which includes proteins containing a region referred as repeats-in-toxin (RTX) [27, 69, 70] and a family of proteins with a signature sequence Proline P-Glutamate E Polymorphic GC-rich Repetitive Sequence (PE_PGRS) [71, 72], the Greek key motif present in the βγ-crystallin superfamily containing Ca^{2+}-binding proteins in Eubacteria and Archaea [29, 73–76] and finally the Big domain motif comprising proteins with an immunoglobulin-like domains [30, 77]. Most of these proteins however, are extracellular proteins and some require Ca^{2+} within the μM to mM range to bind compared to eukaryotic cells that have high Ca^{2+} binding affinity within lower μM to nM range. Nevertheless, reports have shown that cytosolic free Ca^{2+} in *E. coli* can increase to tens of micromolar without any loss of viability, suggesting that bacterial Ca^{2+} targets may have lower affinity for Ca^{2+}.

Prokaryotic CaBP encompass a diverse group of proteins that exhibit great structural variety. Binding of Ca^{2+} may provoke folding to a functional state or may lead to protein stabilization. Structural characteristics of these proteins suggest they may act as buffers, may play a structural role and/or may function as sensors/signal transducers. Much more research is needed to characterize biochemically and genetically bacterial Ca^{2+}-binding proteins offering exciting possibilities and a challenge for the future.

Organism	Protein name	Accession number	a.a. number	EF-hand/ EF-hand-like motif	Potential role of Ca^{2+}	Refs.
Saccharopolyspora erythrea	Calerythrin	P06495	177	Helix-loop-helix	Buffer	[4, 24]
Rhizobium etli	Calsymin	Q9F6V9	293	Helix-loop-helix	Transducer	[4, 36]
Thermotoga maritime	4-α-Glucano-transferase	P80099	441	Helix-loop-helix	Unknown	[4, 24]
Escherichia coli	B Slt35	P41052	361	Helix-loop-helix	Structural	[4, 24]
Bacillus anthracis	Protective antigen	P13423	764	Helix-loop-helix	Structural	[4, 23]
Clostridium thermocellum	Dockerin	A3DCJ4	350	Helix-loop-helix	Structural	[4, 23]
Salmonella typhimurium	Periplasmic galactose binding protein	P23905	332	Helix-loop-strand	Structural	[4, 23]
Sphingomonas sp	Periplasmic alginate binding protein	Q9KWT6	526	Helix-loop-loop	Regulatory	[4, 23]
Pseudomonas aeruginosa	Alkaline protease	Q03023	479	Strand-loop-strand	Unknown	[4, 24]
Halothermothrix	α-Amylase A	Q8GPL8	515	Strand-loop-helix	Structural	[4]

Protein accession numbers in UniProtKB database. Reproduced with permission from Elsevier. Dominguez et al. [4].

Table 1. Examples of bacterial proteins containing EF-hand and EF-hand-like motifs with known structure.

5. Ca^{2+} signaling

The hypothesis that Ca^{2+} acts as a messenger in bacterial cells is based on the observation that environmental signals induce changes in the level of cytosolic free Ca^{2+}. Microorganisms must quickly adapt to changes in the environment in order to survive. Therefore, bacteria must have evolved sophisticated regulatory networks to constantly monitor signals that are critical for their continued existence. How bacterial cells sense the external signal has not been determined yet but experimental observations suggest that may occur through different mechanisms including: cytosolic-free Ca^{2+} transients, membrane sensors, two component systems and its regulatory proteins, and Ca^{2+} sensors transducing the signal.

Over the years, evidence of a Ca^{2+}-mediated stimulus response in bacteria has been documented. Since 1977, Ordal reported that cytosolic Ca^{2+} controlled the rotation of the flagella in

B. subtilis cells. Later work corroborated that cytosolic Ca^{2+} transients affect bacterial motility in *E. coli*, possibly through the phosphorylation of the Che proteins [78–80]. The involvement of Ca^{2+} as a signal transducer in a variety of environmental conditions, where cytosolic free Ca^{2+} is elevated as a result of the stimulus, has been shown in various organisms including: oxidative stress in *B. subtilis* [81], heat/cold shock, and salt and osmotic stress in *Anabaena* strain PCC7120 [14, 82], carbohydrate fermentation products in *E. coli* [19], organic solvents, pharmaceuticals and antibiotics in cyanobacteria [16, 17].

Evidence that membrane-bound proteins may be able to transduce Ca^{2+} signal was shown *in vitro* using the chimeric protein Taz1. Under low concentrations of Ca^{2+}, Taz was phosphorylated leading to the activation of porin genes in *E. coli* [83, 84]. No *in vivo* studies have been followed up. A more recent report in *Vibrio cholera*, showed that Ca^{2+} greatly enhances the transmembrane virulence regulator (TcpP) activity by increasing protein-protein interaction in the presence of bile salts, leading to the activation of downstream virulence factors [10].

Two component regulatory systems, consisting of a sensor kinase and a transcriptional activator, are commonly used by bacteria to sense and respond to environmental signals. Several of these systems have been shown to respond to extracellular Ca^{2+}. In the PhoPQ system in *Salmonella typhimurium* and *P. aeruginosa*, PhoQ is a Mg^{2+}, Ca^{2+} sensor that modulates transcription in response to cation levels. The binding of PhoQ to Ca^{2+}, Mg^{2+} or Mn^{2+} keeps the protein in a repressed state inhibiting the transcription of many virulent genes [85, 86]. In *V. cholera*, the *ca*lcium *r*egulated *s*ensor (carS) and regulator (carR) were shown to be decreased when bacterial cells grew in Ca^{2+} supplemented medium. Further analysis demonstrated that expression of vps (*Vibrio* polysaccharide) genes and biofilm formation are negatively regulated by the CarRS two-component regulatory system [87]. In *V. parahemolyticus*, Ca^{2+} influences gene expression for type III secretion systems ($T3SS_1$) and swarming. A transcription factor called CalR was shown to repress T3SS1 and swarming, which in turn were linked to a σ^{54}-dependent regulator [22]. Another two-component system AtoS-AtoC, which mediates the regulation of PHB complexes in *E. coli* is induced by Ca^{2+}. It was shown that the highest accumulation of PHB complexes occurred in AtoS-AtoC expressing *E. coli* cells compared to deletion mutants AtoSC at high Ca^{2+} concentration in cytosolic and membrane fractions [88, 89]. More recently, in *P. aeruginosa*, the two-component regulator PA2656-PA2657 genes were induced by $CaCl_2$. Deletion mutations and transcriptome analysis revealed that this two-component system may be responsible for regulating the expression of periplasmic proteins and affecting Ca^{2+} homeostasis [90].

Bacterial CaBP that may be involved in in signal transduction include CabC, which may be regulating spore germination and aerial hyphae formation in *Streptomyces coelicolor* [91]. The recently reported EfhP from *P. aeruginosa* that is required for Ca^{2+} homeostasis [38] and other two EF-hand proteins from *S. coelicolor* and *S. ambofaciens* whose function remains to be discovered [92, 93].

Despite all the information accumulated over the past few years, Ca^{2+} signaling in bacterial physiology remains to be elucidated. Further work is needed to uncover the specific nature of the Ca^{2+} signal transduction, its components and their specific regulation and function.

6. Ca^{2+} signals during host-pathogen interactions

Pathogenic bacteria have evolved various strategies to successfully colonize and cause infection in their hosts. Intracellular Ca^{2+} mobilization has been implicated as an important signaling event during bacterial adhesion, invasion and intracellular replication during infection [6]. Interestingly, some pathogens induce Ca^{2+} increases while others interfere with the Ca^{2+} signal to promote invasion [9, 94, 95]. However, despite the significant role of Ca^{2+} signaling during pathogenesis, the mechanisms underlying how bacterial cells and their virulent factors manipulate Ca^{2+} mobilization in host cells remains to be elucidated. This section will present some examples of the role of Ca^{2+} in host-pathogen interactions.

Neisseria meningitidis (meningococci) is the causative agent of bacterial meningitis. Pili are one of the major virulent factors of meningococci. Pili are bacterial structures that play an important role in adhesion to host cells. Analyzing the role of Ca^{2+} during *N. meningitides* infection, Asmat et al. [9] found that the meningococcal protein PilC1 triggered a significant increase of cytosolic Ca^{2+} in human brain microvascular endothelial cells (HBMEC), which was critical for adherence and subsequent internalization into host endothelial cells. Use of the Ca^{2+} chelator, BAPTA-AM, significantly reduced PilC1-mediated meningococcal adherence. Mutants deficient in PilC1 were not able to increase cytosolic Ca^{2+} in endothelial host cells. Pretreatment of host cells with the phospholipase inhibitor, U73122, indicated that the Ca^{2+} increase in endothelial cells was mediated by phospholipase C (PLC). Similar findings where Ca^{2+} mediated adherence to host cells occur through pili were reported in *P. aeruginosa* [96] and efficient internalization via PLC was reported in *Campylobacter jejuni* [97] and *Borrelia burgdorferi* [98].

Shigella is another pathogen that utilizes Ca^{2+} signaling during infection of epithelial cells. *Shigella* is the etiologic agent of bacillary dysentery. This pathogen invades the intestinal mucosa producing massive destruction of the colonic epithelium by eliciting a strong inflammatory response [6]. As early as 5 min after bacterial contact with epithelial cells *Shigella* induces local Ca^{2+} signals in the host cell, which remodel the cytoskeleton allowing bacterial entrance to the cells. Global Ca^{2+} signals are involved in later stages of infection promoting slow cell death as a result of plasma membrane permeabilization and increased cytosolic Ca^{2+}. *Shigella* also manipulates the Ca^{2+} signal to interfere with immune responses and inflammation [99]. Global Ca^{2+} signals have also been associated with an induced decrease of sumoylation by *Shigella*. Sumoylation is a posttranslational modification by *S*mall *U*biquitin *Mo*difier (SUMO) proteins, which is an essential regulatory mechanism involved in several processes including protein stability, cell cycle, cell communication and gene expression [100]. At late time of postinfection, *Shigella* induces inhibition of sumoylation through activation of calpain proteases, which degrade SUMO proteins [101]. Inhibition of Ca^{2+} influx or calpain activity prevented shigella-induced loss of sumoylation. On the other hand Ca^{2+} treatment and inomycin resulted in sumoylation inhibition [101]. Knowledge of how pathogens interfere with SUMO enzymatic machinery is limited and remains to be characterized.

Several bacterial pathogens secrete potent virulence factors such as pore-forming toxins. These toxins perforate host cell membranes in order to deliver virulence factors, escape from

phagosmes or disrupt cell-cell junctions (Tran Van Nhieu [6]; Reboud et al. [102]). Interestingly, some pore-forming toxins such as *Listeria* listeriolysin O (LLO) induce Ca^{2+} oscillations as a result of a direct Ca^{2+} influx via the pore-forming toxin. An interesting feature of LLO is that pores open and close in a synchronized fashion leading to long lasting Ca^{2+} oscillating signaling provoking a broad spectrum of cellular responses during infection [6, 103]. Two other pore-forming toxins produced by the opportunistic pathogens *P. aeruginosa* (ExlA) and *Serratia marcescens* (ShlA), which share similar structural and functional aspects, have the capacity to trigger Ca^{2+} influx leading to disruption of cell-cell junctions of epithelial and endothelial cells. This influx of Ca^{2+} activates a metalloproteinase called 10(ADAM 10), which cleaves cadherin inducing cell-cell-junction breakdown and loss of tissue integrity [102].

There is a great diversity of Ca^{2+}-dependent processes that pathogens utilize to cause infection. However, studies on bacterial induced Ca^{2+} signaling are limited. More research is needed in this filed to understand the mechanisms of how bacterial virulence factors regulate second messengers such as Ca^{2+} and Ca^{2+}-dependent events during the infectious processes.

7. Conclusion

The role of Ca^{2+} in bacteria is a fascinating field that still remains unexplored. It is clear that evidence supporting the role of calcium as a regulator in prokaryotes is accumulating. However, the extent and significance remains unclear. A systematic assessment and careful analysis of the processes involving calcium warrants further analysis.

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Conflict of interest

The author has no conflict of interest.

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