Role of Prokaryotic P-Type ATPases

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Abstract

P-type ATPases is a large and varied family of transmembrane proteins that are responsible for actively pumping ions and small organic molecules, against their concentration gradient, across the cell membrane. They are ubiquitous and reported to be found in bacteria, archaea and eukaryotes. They are responsible for controlling vital functions of the cell like muscle contraction, membrane potential, signaling etc. in eukaryotes. In prokaryotes, relatively few studies have been performed on the biochemical function and in vivo importance of these pumps although a plethora of gene sequences have been obtained from bacterial genome sequences. This review puts together the various roles of P-type ATPases in prokaryotes in which their function has been elucidated. The various roles of P-type ATPases in prokaryotes is to confer on them the ability to withstand high concentrations of heavy metals, to overcome high phagosomal metal levels and to aid in the assembly of periplasmic and secreted metalloproteins. These properties are critically required for their survival in extreme conditions (extremophiles), to withstand heavy metal stress and also for bacterial virulence.

Keywords: Prokaryotic P-type ATPases; Biosensors; Virulence; Heavy metal stress; Vaccine targets

Introduction

Active transport across membranes is an essential feature of life. P-type ATPases establish and maintain steep electrochemical gradients of key cations at the expense of ATP as originally proposed by Jardetzky [1]. P-ATPases (also known as E1-E2 ATPases) (EC:3.6.3) are found in bacteria and in a number of eukaryotic plasma membranes and organelles. P-ATPases function to transport a variety of different compounds, including ions and phospholipids, across a membrane using ATP hydrolysis for energy. There are many different classes of P-ATPases, which transport specific types of ions: H+, Na+, K+, Ca2+, Mg2+, Cd2+ and Cu2+ in species as diverse as bacteria and man [6] and also Zn2+ , Co2+, Au+ and Ag+ which are grouped into the PIB type. Several studies have been made on bacteria such as bacilli [7], pseudomonads [8], Ralstonia spp[9] and cyanobacteria [10]. These studies reveal that the PIB-ATPases regulate concentration of metal ions by export of those which are toxic and import of those which are essential. Phylogenetic analysis between 16S rRNA and PIB-type ATPase gene trees have revealed congruencies pointing to instances of lateral gene transfer (LGT) among diverse microbes. This indicates specific functions for the different clades within the PIB-type ATPase phylogeny [11].

Evolution of Metal Homeostasis in P-Type ATPases as Stress Management Strategy

The cat ionic substrates transported include H+, Na+, K+, Ca2+, Mg2+, Cd2+ and Cu2+ in species as diverse as bacteria and man [6] and also Zn2+ , Co2+, Au+ and Ag+ which are grouped into the PIB type. Several studies have been made on bacteria such as bacilli [7], pseudomonads [8], Ralstonia spp[9] and cyanobacteria [10]. These studies reveal that the PIB-ATPases regulate concentration of metal ions by export of those which are toxic and import of those which are essential. Phylogenetic analysis between 16S rRNA and PIB-type ATPase gene trees have revealed congruencies pointing to instances of lateral gene transfer (LGT) among diverse microbes. This indicates specific functions for the different clades within the PIB-type ATPase phylogeny [11].
simple bacteria have a number of membrane transporters that maintain the homeostasis of the various transition metals. These include, among others, the P-type ATPases [15-17]. P-type ATPases show existence as a single primitive ATPase, prior to the divergence of eukaryotes from prokaryotes [18]. Thereafter, divergence is seen into heavy metal pumping ATPases and the non-heavy metal ATPases as formation of a distinct evolutionary branch [19]. Genes encoding PIB-type ATPases with conserved motifs are found in the majority of sequenced bacterial and archaeal genomes, suggesting several loss and gain events. This corroborates the fact that these are primitive proteins indispensable for their role in life processes of bacteria [11,19] and archaea [19,20]. Such studies on evolution of metal homeostasis genes have significant contribution for comprehending microbial adaptations in environments stressed and continuously changing. The primary function of P-type ATPases in bacteria has therefore been understood as combat against extreme environmental stress conditions as reviewed and suggested by Chan et al.[21].

**Heavy metal stress**

Several studies on the function of PIB type ATPases in efflux of toxic metals like Fe$^{2+}$ in *B. subtilis* [22] and Pb$^{2+}$ in *Staphylococcus aureus* [23] have been made. An extensive exploration into the mechanisms of buffering optimum metal concentrations for cell viability in metal stressed bacteria has revealed the indispensable role of P-type ATPases in efflux mechanisms with a range of substrates (Cu$^{+}$, Zn$^{2+}$, Co$^{2+}$). They can also transport non-physiological substrates (Cu$^{2+}$, Cd$^{2+}$, Pb$^{2+}$, Au$^{+}$, Ag$^{+}$) due to the structural similarities among transition metals [24]. PIB-ATPases not only maintain cytoplasmic metal levels but also provide metals for the periplasmic assembly of metalloproteins [25]. Since PIB-ATPases appear key players in overcoming high phagosomal metal levels and are also required for the assembly of periplasmic and secreted metalloproteins that enable survival in extreme oxidant environments. Copper transporting P-IB type ATPases are the most studied. Genome database analyses have demonstrated that copper translocating PIB-type ATPases are highly conserved in *Staphylococcus aureus* [26].

**Pathogenicity stress**

Stress is also experienced by pathogenic bacteria during establishment of infection in the host cells where they come across change of temperature, pH, cation concentration. Infection not only triggers adaptive responses within bacteria to these specific stress conditions but also directs them to express virulence-associated genes in a spatiotemporally appropriate manner. It has been shown that P-type ATPase in in *Streptococcus pneumoniae* for Ca$^{2+}$- transporting PIB ATPase [27], *Listeria monocytogenes* [28] and enteric pathogen *Salmonella* [29], is vital for survival of the pathogen in the infected host, where Ca$^{2+}$ concentration is very high and must be actively removed from bacterial cell. Transition metal PIB type ATPases have been reviewed and found to play similar role [30]. The role of metal intoxication in host-pathogen interactions was first noted owing to the virulence defects of bacteria that are defective in Zn(II) and Cu(I) efflux [31-34]. Similar studies have been performed for Fe$^{2+}$ and Mn$^{2+}$ intoxication [35-38]. Efflux systems have thus been established to function as virulence factors as also reported in several bacterial pathogens [27-40]. The role of bacterial P-type ATPases has been summarized in Table 1. This shows the great versatility of tasks performed by them.

**Table 1**: Biochemically characterized functions of various P-type ATPases in Prokaryotes.

| S.N. | P-Type ATPase             | Function                                      | Organism              | Reference |
|------|---------------------------|-----------------------------------------------|-----------------------|-----------|
| 1    | P-type Ca$^{2+}$ ATPase   | Virulence                                     | Streptococcus pneumonia | 26        |
| 2    | P-type H$^{+}$-ATPase,    | generation of the primary electrochemical     | Methanooccus jannaschii | 19        |
|      |                            | potential across thermophilic archael membrane.|                       |           |
| 3    | soft-metal-transporting P- | Resistance to cadmium and Zinc                | Ralstonia metalidurans  | 41        |
|      | type ATPases, CaDA and ZnTA|                                               |                       |           |
| 4    | PIB-ATPase                | cobalt, zinc, and cadmium resistance          | Cupriavidus metalidurans | 6,42      |
| 5    | P-type Cd(2+) ATPase      | Cd(2+) extrusion for cadmium resistance       | *Staphylococcus aureus* 17810R | 43        |
| 6    | P-type Na(+)-ATPase       | electrogenic transport of Na(+) in anaerobic   | Exiguobacterium aurantiacium | 44        |
|      |                            | alkaliphile                                    |                       |           |
| 7    | PIB-4-ATPase              | Co$^{2+}$ transport                            | Sulfitobacter sp. NAS-14.1 | 45        |
| 8    | P(1)R-type ATPases        | Cu$^{2+}$ transport                            | Archaeoglobus fulgidus | 30        |
| 9    | CtpA, a copper-translocating P-type ATPase | assembly of membrane and periplasmic copper enzymes | Rubrivivax gelatinnosus | 25        |
| 10   | Ca$^{2+}$-P-type ATPase   | Ca$^{2+}$ extrusion                           | Streptococcus lactis  | 46        |
| 11   | Na$^{+}$-P-type ATPase    | Na$^{+}$ extrusion                            | halotolerant cyanobacterium, Aphanothce halophytica | 46        |
| 12   | copper-transporting P-type-ATPase | Cu$^{2+}$ transport in Anaerobic sulphur-metabolizing hyperthermophilic archa. | Archaeoglobus fulgidus | 47,48,49  |
| 13   | copper-transporting P-type-ATPase | Cu$^{2+}$ transport                           | *Aquifex aeolicus.* | 50        |
Potential Applications of P-Type Atpases

Heavy metal efflux systems enable growth of such heavy metal resistant bacteria in high concentrations of these metals. This property can be potentially harnessed in bioremediation of poorly cultivable soil high in heavy metal content, by precipitation of these heavy metals. Since the regulation of metal resistant gene expression is specific for each heavy metal and is dependent upon metal species concentration, the promoters and regulatory genes from the bacterial operons responsible for resistance can be used to create metal-specific biosensors (promoter-reporter gene fusions) [53]. The recent insights into the role of P-type ATPases in virulence new vaccine strategies that target metal transport systems have been developed. Components of the Zn²⁺ (ZnuD in Neisseria meningitidis) [54] and M⁺⁺ (MntC in S. aureus and PsaA in S. pneumoniae) uptake systems have been identified as potential vaccine targets [55]. Potential drug targets for intervention for the prevention or treatment of infectious diseases in the light of a deeper understanding of host drug targets for intervention for the prevention or treatment of infectious diseases in the light of a deeper understanding of host defense mechanisms have been identified as potential vaccine targets [55]. Potential applications of P-type ATPases in novel drug development.

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