Membrane Proteins as Maxwell's Demons and Their Significance for P Systems

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Summary. The aim of these notes is to contribute to the dialog between P systems and Biological Sciences focussing on membrane proteins involved either in iron transport inside the bacterial cells or in elimination outside the bacterial cells of substances which are dangerous for the cell. The ability of these membrane proteins to behave as Maxwell's demon, gate keeper, or as "a being who can see the individual molecules" could be important for P systems as examples of discrete processes which could be modeled by discrete mathematics and as real molecular objects for *in vitro* implementation of P systems.

1 Introduction

In the framework of the dialog between P systems and Biological Sciences, the aim of this communication is to develop the already increasing interest in Biological Sciences for membrane proteins acting as devices commonly known as Maxwell's demons (Hopfer, 2002; Otsuka and Nozawa, 1998).

The device originally suggested by Maxwell to separate molecules (actually low- and high-speed gas molecules) into different compartments has as a crucial element an intelligent gate keeper (Hopfer, 2002), called by Maxwell himself "a being, who can see the individual molecules" (Maxwell, 1871), a being which was called later on Maxwell's demon. The mechanism was originally described as follows: "Now let us suppose that such a vessel is divided into two portions, A and B, by a division in which there is a small hole, and that a being, who can see the individual molecules, opens and closes this hole, so as to allow only the swifter molecules to pass from A to B, and only the slower molecules to pass from B to A (Maxwell, 1871).

In this paper, in order to contribute to the dialog between P systems and Biological Sciences, we will focus on membrane proteins involved in iron transport

inside the bacterial cells and on other membrane proteins involved in elimination outside the bacterial cells of substances which are dangerous for the cell (antibiotics and solvents), the so-called multidrug resistance (MDR) efflux systems.

We also put forward the question if Maxwell could be seen as a precursor of membrane computing because he imagined nanosized devices working at the boundary between two compartments.

These membrane proteins involved in iron transport inside the bacterial cells and in the elimination outside the bacterial cells of substances which are dangerous for the cell (antibiotics and solvents) could have significance for P systems in the followings:

a) as examples of discrete processes which could be modeled by discrete mathematics;
b) as real molecular objects for *in vitro* implementation of P systems.

2 Membrane Proteins Involved in Iron Transport Inside the Bacterial Cell

Iron is a crucial microelement in microbial metabolism, playing a vital role in many important biological processes such as respiration and biomineralization. Biomineralization, the process by which organisms transforms soluble substances into mineral crystals, plays a significant role in environmental iron cycling, the magnetization of sediments and thus the geologic record, and in the use of biomarkers as microbial fossils (Bazylinski et al., 2007; Simmons et al., 2004; Stolz, 1990; Petersen et al., 1986). Magnetotactic bacteria (MTB) can be considered as a model system for biomineralization of iron oxide and sulfide nanocrystals produced by living organisms. MTB are a fascinating group of microorganisms (Blakemore, 1979; Schüler and Baeuerlein, 1998; Ignat et al., 2007; Ardelean et al., 2008; Logofătu et al., 2008), which exhibit the peculiar ability to orient themselves along the magnetic field lines of Earth's magnetic field. The sensitivity of MTB to the Earth's magnetic field arises from the fact that the bacteria precipitate within their cells chains of lipid membrane-enclosed crystals of magnetic minerals magnetite (Fe_3O_4), greigite (Fe_3S_4), or both, referred to as "magnetosomes", which serve as a navigational device for spatial orientation in marine and freshwater habitats. In MTB, a magnetic particle synthesis system was proposed (Mann et al., 1990), which involves the following discrete processes occurring across and within biological membranes: (i) uptake of iron, (ii) transport of iron to the cytoplasm and across the magnetic particle membrane, (iii) precipitation of hydrated ferric oxide within vesicles, and (iv) phase transformation of the amorphous iron phase to magnetite, during both nucleation and surface-controlled growth.

Recent molecular studies have postulated the steps of bacterial magnetic particles (BMP) synthesis in *Magnetospirillum magneticum* strain AMB-1 (Okamura et al., 2001; Matsunaga et al., 2000; Nakamura et al., 1995a). The presentation of these steps in some detail could help the mathematicians and informaticians

to deeper accommodate with the progresses at molecular level in this hot topic of nowadays Microbiology and, hopefully, to find inspiration for other application of these proteins in P systems, than those proposed by us, pure microbiologists, in this communication.

1. The first event of BMP synthesis is the formation of vesicles (magnetosome membrane), which further argues the role of membranes in biomineralization. Okamura et al. (2001) identified a 16 kDa protein, called Mms16, which was the most abundant of the magnetosome specific proteins in *M. magneticum* AMB-1. The Mms16 protein was confirmed to be expressed only on the BMP membrane, thus Okamura and coworkers were the first to report the experimental function of a BMP-specific protein. Their results also suggested that this novel Mms16 protein, specifically localized on the magnetic particle membrane, is a GTPase, being able to split GTP, a compound rich in useful energy for the cell. The proposed mechanism supposes that the Mms16 first binds with the cytoplasmic membrane. This binding serves to prime the invagination of the cytoplasmic membrane for the intracellular vesicles formation, which will become the future BMP membrane. Acyl-CoA and GTP hydrolysis might be required during vesicle budding. Therefore, another magnetosome specific protein, called MpsA, a homolog of an acyl-CoA carboxylase (transferase) containing a CoA-binding motif, is also considered to be involved in this process (Matsunaga et al., 2000) functioned as mediator for BMP membrane invagination (Matsunaga et al., 2000), but its exact function remains unclear.

2. The second process in BMP synthesis is iron transport (see Figure 1). Iron exists in two redox states: the reduced Fe^{2+} ferrous state and the oxidized Fe^{3+} ferric form. In natural environments, iron predominantly occurs as ferric iron (Fe^{3+}) under aerobiosis. Fe^{3+} as iron hydroxide is poorly soluble in aqueous solution, rendering it basically unavailable for the cells (Neilands, 1981). Under anaerobiosis, reducing or acidic conditions, the iron equilibrium shifts from the ferric Fe^{3+} to the ferrous Fe^{2+} form that is more easily available for microorganisms. Thus, several aspects of the general system needs to be made clearer, for example, whether the ferric or ferrous ion is taken up and transported and which proteins control the reactions in each stage. The studies of Suzuki et al. (2006) showed that a robust ferrous ion-uptake system coupled to magnetosome synthesis exist within *M. magneticum* AMB-1. It appears that in *M. magneticum* strain AMB-1 ferric iron is reduced on the cell surface, taken as ferrous iron into the cytoplasm, transported into the BMP vesicle, and finally oxidized to produce magnetite (Arakaki et al., 2003) which is an interesting system to be modeled within the framework of membrane computing. The later studies of Suzuki et al. (2007) also revealed that the activity of this ferrous ion-uptake system is modulated by a cytoplasmic ATPase (gene product of ORF4 operon), which accelerates the uptake of ferrous ions through the cell membrane into the cytoplasm by energizing cell membrane ferrous ion transporters FeoAB, Tpd and Ftr1 (see Figure 1).

There are several families of proteins involved in iron transport in Prokaryotes. These families are grouped in two main groups: ferrous ion (Fe^{2+}) transporters,

and ferric ion (Fe^{3+}) transporters. From the first group, the following genes (and consequently the proteins encoded by these genes) are present in *M. magneticum* AMB-1: *ftr1, tpd, feoA*, and *feoB*. From the later group, the *cirA, fepA, fepC, tonB, exbB, exbD, tolQ, napA, napB*, and *napC* genes (respectively proteins) are present in *M. magneticum* AMB-1 (Suzuki et al., 2006).

The *ftr, tpd,* and *feo* genes are known to be expressed under low-oxygen conditions when ferrous iron remains stable and predominates over ferric iron (Andrews et al., 2003; Dubbels et al., 2004; Felice et al., 2005; Kammler et al., 1993; Marlovits et al., 2002). The studies of Suzuki et al. (2006) also revealed that in *M. magneticum* AMB-1 the ferrous iron transport system is triggered under reducing conditions (with low-oxygen levels) these results being consistent with the microaerobic culture conditions in which the cells were grown. On the other hand, ferric ions transport genes, which include *fepA, tonB, exbB*, and *exbD* (Andrews et al., 2003), were downregulated under iron-rich conditions. Additionally, higher transcript levels of nitrate reductase (amb2686, amb2687, amb2690) and ferric reductase (amb3335) genes were obtained under iron-rich, magnetosome-forming conditions (Suzuki et al., 2006).

3. The last process is crystallization of magnetite within the intracellular vesicles (magnetosome membranes). Several proteins appear to be required for magnetite crystallization and the first reported protein was MagA, isolated from *M. magneticum* AMB-1 (Nakamura et al., 1995a). Internal localization analysis of the MagA protein indicated that, unlike Mms16 protein, MagA is localized on both cytoplasmic membrane (Nakamura et al., 1995b), and BMP membrane and showed iron transport activity. Interestingly, MagA topology is inversely oriented between the cytoplasmic membrane and the BMP membrane (Nakamura et al., 1995a; 1995b), something which would occur if magnetosomes were formed by membrane invagination. MagA appears to function for iron efflux in the former and iron influx in the latter. The number of MagA molecules per magnetosome volume is much larger than per cell volume as calculated from the total amount of expressed MagA (Nakamura et al., 1995b). This makes the quantity of effluxed iron by MagA on the cytoplasmic membrane negligible. The iron-uptake activity of MagA was determined using inverted vesicles prepared from fragmented membrane-expressing MagA protein in *E. coli*. Addition of ATP initiated the accumulation of ferrous ions in the vesicles. The ions were released by the addition of carbonyl cyanide m-chlorophenylhydrazone (CCCP), also known as protonophore (Nakamura et al., 1995a). This activity was also observed under an artificial proton gradient without ATP. These results suggest that MagA protein is a proton-driving H^+/ Fe^{3+} antiporter (Matsunaga et al., 2000). MagA protein may play roles in transporting Fe^{3+} to the vesicle to grow up to BMPs and the alkalization of the inside of vesicles due to its H^+/ Fe^{3+} antiporter function.

Furthermore, Mms6 protein isolated from BMP membrane was shown to function for the crystallization of ferric and ferrous ions under anaerobic conditions (Arakaki et al., 2003). Magnetite crystallization is inorganically derived, as demonstrated by Frankel et al. (1983), but Mms6 directly binds ferric iron and regulates

crystallization and morphology during magnetite formation in *M. magneticum* AMB-1 (Arakaki et al., 2003), acting as an organic matrix for crystal formation.

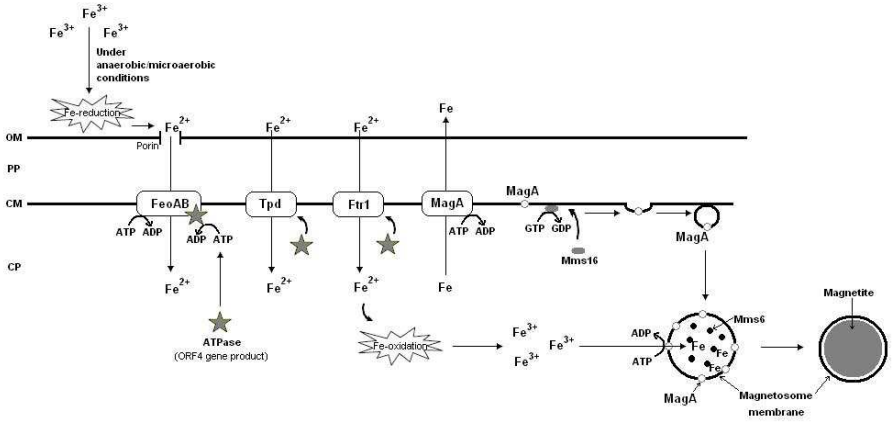

Fig. 1. Schematic representation of the possible ferrous ion uptake system during magnetosome synthesis within *M. magneticum* AMB-1 strain (adapted after Suzuki et al., 2007; Matsunaga et al., 2004). The Mms16 protein attaches to the CM initiating the intracellular vesicle formation (first step in magnetosome synthesis). Under the microaerobic growth conditions, the extracellular Fe^{3+} is reduced on the cell surface to Fe^{2+}, thus making the iron available for the microorganism. An ATPase (the ORF4 gene product) present in the cell cytoplasm, accelerates the uptake of the Fe^{2+} ions through the cellular membrane into the cytoplasm, by energizing cell membrane Fe^{2+} transporters FeoAB, Tpd, and Ftr1. The transported Fe^{2+} is then oxidized to Fe^{3+} in the cytoplasm, and transported into the intracellular vesicles (the future magnetosome membrane) by the MagA, a H^+/Fe^{3+} antiporter protein. The Mms6 protein present inside the magnetosme vesicles binds Fe^{3+}, and acts as an organic matrix for magnetite crystal formation in *M. magneticum* AMB-1. OM, outer membrane; PP, periplasm; CM, cytoplasmic membrane; CP, cytoplasm.

3 Multidrug Resistance (MDR) Efflux Systems

The efflux of the hydrocarbons by multidrug resistance (MDR) efflux systems is the most important mechanism of hydrocarbons tolerance in bacteria used for maintaining the hydrocarbons concentration in the cell under its equilibrium level. Together with MDR efflux systems microorganisms can use other mechanisms to resist toxic hydrocarbons such as: metabolism of toxic hydrocarbons, which can contribute to their transformation into nontoxic compounds; rigidification of the cell membrane via alteration of the phospholipids composition; alterations in the cell surface that make the cells less permeable; formation of vesicles that remove the

solvent from the cell surface; and efflux of hydrocarbons in an energy-dependent process (Ramos et al., 2002; Segura et al., 1999, 2007).

MDR efflux systems catalyze the active extrusion of many structurally and functionally related and unrelated compounds from the bacterial cytoplasm (or internal membrane) to the external medium (Segura et al., 1999; Ramos et al., 2002). Some of the substrates of these MDR pumps are hydrocarbons that do not resemble any of the known natural substrates that these cells may have encountered during evolution. The data available indicate that it is the physical characteristics of the compounds (e.g., charge, hydrophobicity or amphipathicity), the Van der Waals interactions they establish with active sites and effectors pockets, and the flexibility of these sites in the target proteins that determine the specificity of these multidrug efflux systems (Neyfakh, 2001).

Multidrug efflux systems have been the subject of recent reviews, and five main families of MDR transporters have been identified in bacteria. These include: 1) the major facilitator superfamily (MFS); 2) the ATP-binding cassette (ABC) family; 3) the resistance nodulation and cell division (RND) family, which is part of the larger RND permease superfamily; 4) the small multidrug resistance (SMR) family; and 5) the multidrug and toxic compound extrusion (MATE) family. The most well characterized representatives of these families from Gram-positive and Gram-negative bacteria are shown in Figure 2A and 2B, respectively. Small multidrug resistance (SMR) and multidrug and toxic compound extrusion (MATE) look structurally similar with the major facilitator superfamily (MFS) but are designed as distinct families, based on size (i.e., SMR) or phylogenetic diversity (i.e., MATE). All of these transporters catalyze active drug efflux and therefore require energy, mostly in the form of proton motif force (i.e., utilize the H^+ or Na^+ transmembrane electrochemical gradient for pumping the antibiotics or other compounds from the inner to the outer space of the cell), or in the form of ATP (i.e., utilize the release of phosphate bond-energy by ATP hydrolysis for pumping the antibiotics or other compounds from the inner to the outer space of the cell) (Putman et al., 2000; Schweizer, 2003).

The efflux pumps for antibiotics and hydrocarbons work with exceptional efficiency in Gram-negative bacteria due to synergistic action of cytoplasmic membrane with outer membrane. In Gram-positive bacteria, the efflux pumps move the substrate across just one membrane. This is rather inefficient, as they have to compete with the rapid spontaneous influx of the lipophilic molecule back into the cytoplasm. A high rate of efflux is therefore required to produce significant levels of resistance. The efflux pumps in the Gram-negative bacteria traverse both the cytoplasmic and outer membranes. As the outer membrane is composed largely of lipopolysaccharides (LPS), it has different permeability properties compared to the membrane of Gram-positive bacteria. The membrane of Gram-negative bacteria allows the penetration of lipophilic molecules but at a rate 50-100 times slower than the phospholipid bilayer of Gram-positive bacteria. The decrease in penetration of lipophilic molecules is responsible for the intrinsic resistance of Gram-negatives to certain antibiotics and hydrocarbons. Hydrophilic molecules enter in the mem-

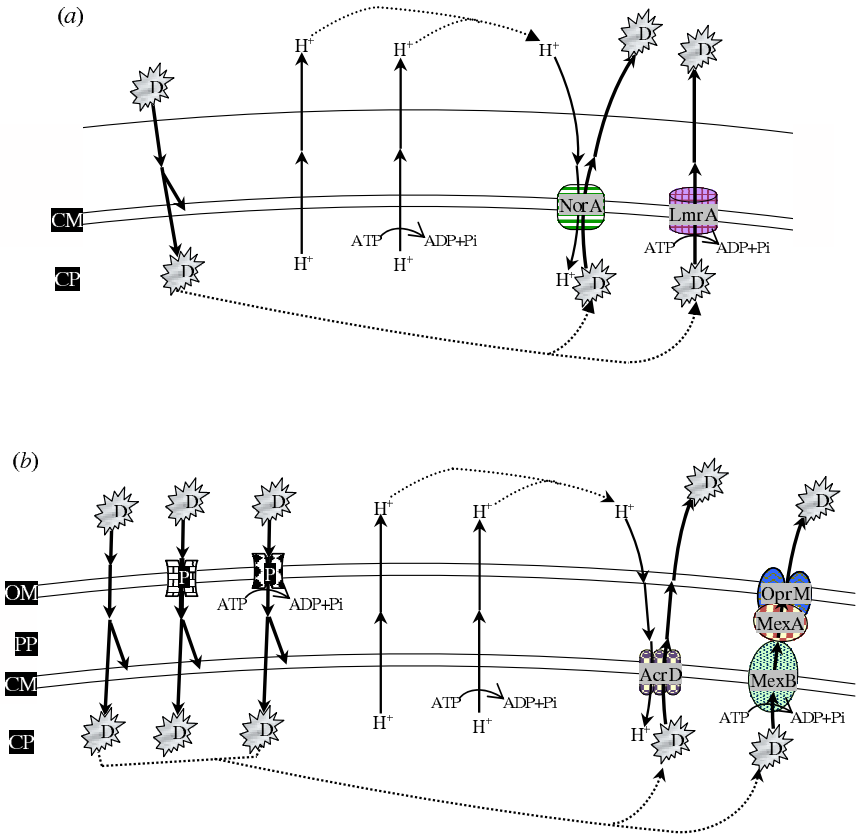

Fig. 2. Schematic illustration of the main types of bacterial drug efflux pumps in Gram-positive (a) and Gram-negative (b) bacteria. (a) Illustrated are NorA, a member of the major facilitator superfamily (MFS); LmrA, a member of the ATP-binding cassette (ABC) family; (b) AcrD and MexAB-OprM, two memers of resistance nodulation and cell division (RND) family. D, drug; OM, outer membrane; PP, periplasm; CM, cytoplasmic membrane; CP, cytoplasm; P, porin.

brane of Gram-negative cells through porins, but in the presence of hydrophilic molecules (e.g., antibiotics, hydrocarbons) or when efflux mechanisms are induced, a decrease in the number of porins in the membrane is also seen. This leads to decreased penetration of the hydrophilic molecules (Nikaido, 1998, 2003).

The intellectual framework of metabolic engineering is built upon the integration of biological information in an attempt to induce higher order principles that govern cell behavior. As such, metabolic engineering and the emerging field of systems biology share an over-arching emphasis on revealing general biological principles from the analysis of the regulation and activity of biological networks ranging from gene sequence to gene expression to metabolic flux (Stephanopoulos

and Gill, 2000). The engineering of new traits or re-engineering of existing traits, which is dependent upon such biological networks, is a major thrust of metabolic engineering. Specifically, metabolic engineering involves the modification of the genetic makeup of an organism in an attempt to re-direct cell behavior in a specific manner. This might involve, for example, engineering increased or decreased expression of a gene that is thought to influence production of a valuable chemical product. While such an example may appear to be straightforward, it is complicated by the fact that metabolism forms a network of chemical reactions that are mutually interdependent and that incommensurately influence overall network activity, which itself influences the relative fitness of an organism in a particular environment. Therefore, any attempt to engineer flux through a specific pathway in an organism can result in secondary effects that may include a reduction in the overall fitness of the organism and, as a result, reduce the attractiveness of the engineering strategy). Antibiotic resistance provides numerous examples of how nature has approached this problem. In particular, antibiotic resistance exemplifies how bacteria routinely develop new phenotypes through combinations of creative and hard to predict mechanisms and the importance that environment plays in selection and maintenance of such phenotypes. As such, it serves as a model to elucidate underlying evolutionary mechanisms that might be applied to the development of future metabolic engineering efforts (Bailey, 1991; Bailey, 1999; Bonomo and Gill, 2005).

In our opinion, the development of future metabolic engineering of MDR efflux systems would benefit if they would be studied in the framework of P systems which could deeply take into account the discrete nature of these proteins working at/within the biological membranes

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