On Modelling Ion Fluxes Across Biological Membranes with P Systems

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Summary. In this report we address the challenge of using P systems to integrate at the whole cell level both *active* and *passive* transport of different ions, done by different types of membrane transport proteins which work simultaneously and concurrently.

1 An Introduction and Some General Considerations

P systems, proposed in [17] and lately reviewed in [18], are a class of distributed and parallel computing devices inspired by the structure and the functioning of living cells. The basic model consists of a cell-like membrane structure, composed by several compartments where multisets of objects evolve according to given rules, in a nondeterministic and maximally parallel manner. A survey and an updated bibliography of P system works is available at http://psystems.disco.unimib.it.

These systems were not initially intended to be a model of the cell, indeed many research studies about P systems focus on computational power aspects. Anyway, in several recent works the framework of P systems has been used to define models of specific cellular processes or structures, as well as to analyze complex biological systems, with the final goal of producing new tools and acquiring useful information about the modelled system. For instance, in [5] mechanosensitive channels in prokaryotes were analyzed, by first defining a P system that models in details their functioning during patch clamping experiments, and then simulating that system by means of the complex system simulator *EdnaCo* [11]. Similar approaches have been followed also in, e.g., [6], where bacterial processes of respiration and photosynthesis are simulated; in [19], where continuous P systems are used to describe the EGFR signaling pathway; in [20], where dynamical probabilistic P systems

are used to analyze the behavior in the phase space of the Belousov-Zhabotinskii reaction (see also [21] for other applications).

Thus, it is clear that P systems have entered the research direction of modelling biological systems, a challenge that is getting more and more important as one can see from the explosion of Systems Biology area (see [14, 12] for a comprehensive introduction to the topic). According to the general guidelines of Systems Biology, in short, one starts from the biological knowledge and data available on a bio-system, builds a mathematical model for that system and then simulates it by means of an appropriate software, with the final goal of analyzing the peculiar aspects (e.g., robustness) and behaviors (stable or unstable states, attractors, etc.) of the system, predicting unknown features and designing further specific experiments aimed at a better understanding of the system itself. In this direction, P systems can represent a novel and powerful methodology, due to the following - among others - relevant characteristics:

- they are discrete systems, that is finite multiplicities of objects (representing biochemical substances) are usually considered instead of continuous values (concentrations). This approach can be considered better for modelling stochastic processes occurring in finite volumes, as cellular compartments are;
- they have an intrinsic parallelism, at the level of rule application and objects consuming, instead of the sequential way of proceedings of many other modelling approaches for biological systems. The parallelism makes P systems closer to the biological reality, where not only one reaction take place in any fixed time, but many different processes can occur, simultaneously and concurrently. Moreover, also some kind of *bounded* parallelism can be considered as well, when appropriate (see [19]);
- they are very flexible, thus allowing local and global descriptions of a biological system. For instance - related to ion fluxes - one can model the *local* structure and functioning of an ion transport protein (see [7]), hence its conformations, the passage of ions, the rate constants associated to transitions between conformations and so on, but also one can analyze the *global* dynamics of fluxes (see [20] for tools and [5] for simulations);
- they are easy understandable (maybe more than models based on differential equations) even to non experts in the field: the hierarchical arrangements of compartments and the form of rewriting rules easily allow the graphical description and the logical separations of substances and processes, according to the cellular counterparts.

Concerning the modelling of ion fluxes across biological membranes, it was previously shown in [5] that P systems can be used to model passive transport of ions and molecules across plasma membrane, namely the transport facilitated by mechanosensitive channel of large conductance (MscL, in short). In this report we address a new challenge: the potentiality of P systems to integrate at the whole cell level both *active* and *passive* transport of different ions, done by different types of transport proteins which can work simultaneously and concurrently.

In the next section we present several problems related to the modelling of transport processes in biological systems: we describe a few specific ion exchanges and also point out why they are important for the cell, whether their dynamic is influenced by other cellular processes or viceversa, and so on.

2 Ion Fluxes Across Biological Membranes

Biochemical substances like gases and small uncharged molecules (urea, ethanol) can directly cross the cellular membranes by passive diffusion, down their concentration gradients. On the contrary, the biological membranes are not permeable to most water-soluble molecules and ions, whose transport is selectively mediated by transport proteins associated with the phospholipid bilayer. The three major types of transport proteins are ATP-powered pumps, ion channels and transporters (uniporter, symporter, antiporter), which all exhibit a high specificity for the transported substances but differs for the rate of transport and the mechanism of action:

1. *ATP-powered pumps* (or ATPases) use the energy of ATP hydrolysis to move ions or small molecules against a chemical concentration gradient or electrical potential (this process is known as *active* transport);
2. *ion channels* simultaneously transport multiple water molecules or many (specific) ions down their concentration or electric potential gradients, at a very rapid rate. Some of them are usually open (for instance, the potassium-specific channel), others are usually closed and open only in response to specific signals;
3. *transporters* bind only one (or a few) molecules at the same time, then a conformational change of the protein allows the transport of these molecules across the membrane. Among transporters, *uniporters* move one molecule at a time down its concentration gradient, while *symporters* and *antiporters* couple the passage of one type of molecule (or ion) against its concentration gradient to the passage of a different type of molecule (or ion) down its concentration gradient.

For more notions about membrane transport proteins see [15, 3, 4].

Correlation between *in vitro* and *in vivo* data. In the last decade the *in vitro* approaches significantly advanced but there are much less available data concerning *in vivo* function of a given transport protein. Practically, no data are available at all about the interaction at cellular level among different types of transport proteins. For instance, much work has been done on the molecular knowledge of MscL in *E.coli*, but very little is known about the interplay between MscL and other types of mechanosensitive channels in *E.coli* for the facilitated transport of ions (sodium, potassium, etc.) or molecules (glucose, etc.). The situation is even worse for the interplay between mechanosensitive channels and other transport proteins involved in the passage of the same molecules and ions outside the cell (see below for some examples).

As parallelism is an intrinsic property of P systems, it is expected that they will be useful to model the interplay based on simultaneity of transport processes, as they were already successful in modelling the *in vitro* function of a single MscL (extensible to the modelling of a population of MscL), and even to propose a model for *in vivo* function of MscL [5].

Transport of the same type of ions by different mechanisms. Another aspect of parallelism occurring at the cellular level is the transport of one given type of ion (or molecule) by different mechanisms. For example, in Cyanobacteria living at low salinity levels (corresponding to tap water, up to 50mM NaCl) and at an elevated pH (above 8.0 units) there are several routes for sodium ions to enter from the extracellular compartment inside the cytosol: (*i*) sodium/solute symporters; (*ii*) cation channel; (*iii*) pH gated sodium channel. Furthermore, the extrusion of sodium ions from the cytosol to outside the cell can take place by two others ways: (*iv*) ABC transporters; (*v*) Na^+/H^+ antiporters (for more details see [22]).

In situations like these, one could easily integrate different small models based on the transport modes (*i*)–(*v*) into a unique P system, and then run some simulations to establish the global behavior of sodium fluxes inwards and outwards the cell. Moreover, several copies of each small model can be run in parallel, in order to have a closer resemblance to reality, or else silenced, if one wants to investigate particular conditions where one or more types of transport are not (correctly) functioning.

Transport of the same type of ions by the same mechanism but with different transporters. In this case, one given type of ion (or molecule) is transported by the same mechanisms but by different transporters. This happens, e.g., in *E.coli*, where two types of Na^+/H^+ antiporters are involved in maintaining the pH of the cytoplasm around a value of about 7.5. Na^+/H^+ antiporters are membrane proteins that exchange Na^+ (or Li^+) for H^+ (reviewed in [16]), which are involved in the maintenance of a relatively constant concentration (homeostasis) of these two types of ions inside the cell. When the external pH shifts from 7.2 to 8.3, the intracellular pH shortly increases to 8.3 and then the antiporter opens and increases the passage of both ions: the protons come inside the cell and the sodium ions are extruded. The movement of both type of ions contributes to the acidification of the cytoplasm that, indeed, returns to its normal pH of about 7.5.

The mechanism by which *E.coli* acidifies its cytoplasm to maintain the pH value is not fully understood, but Na^+/H^+ antiporters play a role in this process. Two genes encoding Na^+ (and Li^+) specific antiporters were identified in *E.coli*, *nha*A and *nha*B. The gene *nha*A encodes a protein, that is the main antiporter, which is required to withstand the upper limit concentration of Na^+ for cell growth (0.9M NaCl, pH 7.0) and to tolerate the upper pH limit for growth in the presence of Na^+ (0.7M NaCl, pH 8.5). The gene *nha*B encodes a protein that acts as a housekeeper and becomes essential only in the absence of *nha*A gene. The experiments have shown that the increase in Na^+ and Li^+ concentrations are the environmental

signal which turns on *nha*A gene; alkaline pH potentiates the effect of these ions but neither increase of osmolarity nor of ionic strength induce this gene. Further results showed that the intracellular Na^+ concentration is actually the direct signal for the transcription of *nha*A gene. These results demonstrate for the first time that *E.coli* has a unique regulatory network responding specifically to Na^+ and Li^+ [16].

A P system model ranging from the interactions at genetic level to ion fluxes (that is, a gene regulatory network at many level of hierarchical complexity in the cell) could hopefully help in clarifying such mechanisms via stochastic descriptions and software simulations.

Functions of ions inside the cell. Related to the above point, one given type of ion (or molecule) always has more than one function into a given cell belonging to a given strain. For example, protons in *E.coli* are involved in: (*i*) the formation of the electrochemical gradient of protons; (*ii*) ATP synthesis; (*iii*) motility; (*iv*) different types of secondary transport (antiport, symport), and more. In Cyanobacteria protons are involved also in respiration and photosynthesis: protons are pumped outside the cell membrane during the respiration and pumped inside the intracellular membrane (thylakoide membrane) during photosynthesis. Respiration is the biological process that allows the cells to obtain energy: in short, respiration promotes a flux of electrons from electron donors to a final electron acceptor which, in most cases, is molecular oxygen. Thus, during the last step of respiration water is formed from molecular oxygen, protons ($4H^+$) and electrons ($4e^-$), and 4 protons are simultaneously transferred across membrane from inside to outside the cell contributing to energy conservation. The overall process of photosynthesis consists in using electrons from water to ultimately reduce carbon dioxide, thus forming substances such as carbohydrates, for example. This process is essential for the life on Earth, being the main energy source for almost all living cells, including human cells, the only source of molecular oxygen needed for respiration (and many oxygen-consuming related activities) as well as a huge carbon dioxide-consuming process. The first major event in photosynthesis is the splitting of water (at the expense of light energy, not presented here for the sake of simplicity) to molecular oxygen, protons and electrons. For each molecule of water 2 protons appear inside the intracellular membrane and during the following reaction light other 8 protons are moved from the cytoplasmic space across intracellular membrane to the internal thylakoidal space. Thus, the cytoplasm is a reservoir of protons (actually, ions of hydrogen) from where the protons are pumped outside the cell in respiration and inside the thylakoides during the photosynthesis.

From the point of view of P systems, this case reflects the previous ones, but with a further interesting aspect: the interplay between two distinct compartments - plus the extracellular space - involved in ion fluxes and in the regulation of cell activities. A good simulation of ion fluxes should then consider all the involved physical spaces, and their respective communications.

Cellular localization of ions. The picture for the cellular localization of only one type of ion into a given cell, belonging to a given strain, is far from being fully understood. Just to give a famous example, the generation of the electrochemical gradient of protons *across* the plasma membrane determined by the electron transport *within* the plasma membrane was explained by the *chemiosmotic theory* (by P. Mitchel, Nobel Prize, 1976). Mitchel thought that the electrochemical gradient of protons is distributed homogenously across the plasma membrane. This view received serious criticism and another model, based on the so-called *localized proton gradient*, proved the opposite with some experimental evidence concerning localized gradients at the level of discrete sites of the plasma membrane.

Moreover, in the case of transport proteins, the rate and extent of ion transport is influenced by the ion concentrations on the external and internal sides of the membrane, and by the electric potential that exists across the membrane. Since the concentration of ions or molecules is not uniformly distributed all over the cellular membrane (there can exist small local area with a higher concentration with respect to others with a lower one), some elements in the population might be more active, others working at lower rates, others even resting according to the position of each (type of) transport protein in such areas and also to the respective position of the surrounding transport proteins (of the same or different type). For instance, in eukaryotic cells one can consider the possibility of analyzing a population of channels of the same type (e.g., a population of sodium-potassium ATPases) or of different types, possibly "competing" for the transported molecules ions (e.g., a population of calcium ATPases, calcium-sodium antiporters, sodium-potassium ATPases, potassium channels).

To define appropriate models for simulating the behavior of channels under different local conditions, we believe it might be useful to approach this type of analysis by integrating P systems and fuzzy techniques (see [1, 2] for some preliminary discussions in this direction).

3 Final Remarks

It is well known in Biology that ion fluxes across the membranes can drive many cellular processes, as briefly explained in Section 2 and well documented in [13] for, e.g, calcium dynamics. It turns out that the investigation of the global behavior of populations of transport proteins and the dynamics of the corresponding transported molecules is a very important topic in Biology, especially in studies aimed at an holistic understanding of biological systems (see [9] for a review of many modelling techniques and examples from the molecular to the cellular level).

P systems were defined as a class of parallel and distributed computing devices, and lately it was shown that they can be also applied to the modelling of cellular or chemical processes. A better understanding of the potentialities of P systems, as well as their limits, would make them a feasible novel methodology in Systems Biology, thanks to their many relevant structural features. Then, the development

of a software simulator based on P systems, integrated with some tools for dynamics analysis and an input/output graphical interface, could eventually allow biologist to create and run their own models.

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