

## **Emergence of Membrane Bioenergetics from Ancient Systems of Na<sup>+</sup>/K<sup>+</sup> Homeostasis**

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It is well known that the cytoplasm of living cells, generally, contains more potassium ions than sodium ions. The evolutionary importance of the potassium prevalence in the cytosol had been first noted by Archibald Macallum in his seminal paper where he formulated the principles of evolutionary chemistry of living organisms (paleochemistry) [1]. This trait seems to be universal; there is overwhelming evidence that K<sup>+</sup> levels in all actively growing cells are substantially higher than the levels of Na<sup>+</sup> ions, see [2] and references therein. It is well established that prevalence of K<sup>+</sup> ions is crucial for the activity of numerous (nearly) universal, key enzymes, including those components of the translation system that even preceded the Last Universal Cellular Ancestor (LUCA) [2-4]. In those experiments where Na<sup>+</sup> and K<sup>+</sup> ions were both included in the reaction mix for K<sup>+</sup>-dependent cellular systems, Na<sup>+</sup> ions have been shown to act as inhibitors [4, 5].

The inhibitory effect of Na<sup>+</sup> on many of these ubiquitous K<sup>+</sup>-dependent enzymes does not seem compatible with the evolution of the respective cellular systems in environments with high sodium levels. Based on the data on the prevalence of potassium over sodium in cellular tissues, Macallum suggested that the first cells might have emerged in K<sup>+</sup>-rich habitats [1]. Different, albeit complementary, scenarios have been recently proposed for the primordial K<sup>+</sup>-rich

environments based on experimental data and theoretical considerations [3,6,7]. Specifically, building on the observation that the  $[K^+]/[Na^+]$  ratio is much greater than unity at vapor-dominated zones of inland geothermal systems, we argued that the first cells could have emerged in the pools and puddles at the periphery of primordial anoxic geothermal fields, where the elementary composition of the condensed vapor would resemble the internal milieu of modern cells [3,8].

In modern prokaryotic cells, the  $[K^+]/[Na^+]$  ratio  $> 1.0$  is maintained by ion-tight cellular membranes and an arsenal of ion pumps. It is unlikely that modern-type ion-tight membranes made of two-tail lipids, not to mention a plethora of ion-pumping machines, were present in the very first cells [9,10]. It is more likely that the monovalent ion content of the cytoplasm of the first cells would have to be equilibrated with the environment. Marine and freshwater environments generally show a  $[K^+]/[Na^+]$  ratio less than unity. Therefore, to invade such environments, while maintaining the cytoplasmic  $[K^+]/[Na^+]$  ratio over unity, primordial cells needed ion-tight membranes and means to extrude sodium ions. The foray into new,  $Na^+$ -rich habitats was the likely driving force behind the evolution of diverse redox-, light-, chemically-, or osmotically-dependent sodium export pumps as well as the increase of membrane tightness, see [2,9,12] for details. One of such export pumps was the  $Na^+$ -translocating rotary ATPase, the relic of which, the so-called N-ATPase, was identified by phylogenomic analysis and suggested to be a  $Na^+$ -export pump [13]; later it was shown to impart halotolerance to fresh-water cyanobacteria indeed [14]. At some point, under the conditions of high salinity of the primordial ocean, the  $Na^+$ -translocating rotary ATPase, which initially functioned as a sodium export pump, could change the direction of rotation and start to synthesize ATP at the expense of the transmembrane sodium potential, thus launching the membrane bioenergetics [9]. In a rotary ATP synthase the energy of sequentially translocated ions is stored stepwise in the elastic deformation of the enzyme until enough energy to drive ATP synthesis is accumulated [15]. Hence, the rotary ATP synthase is a unique machine that can synthesize ATP by accumulating small portions of energy, which could be harvested by diverse redox- or light-driven ion translocases [2,16]. Furthermore, the rotary ATP synthases could be driven even by expelling

“garbage” out of the cell - when the expelled cation(s) is/are accompanied with anionic forms of the end-products of cell fermentation, such as acetate or lactate [17,18]. On the primordial anoxic Earth, organic acids should have been the end-products in most of prokaryotic fermentation pathways; the respective symporters should have been widespread and capable of generating membrane potential for ATP synthesis (almost) “for free”.

With further evolution of cell membranes and after the oxygenation of the atmosphere, the enzymes of membrane bioenergetics gradually became decoupled from the machinery responsible for maintaining the  $\text{Na}^+/\text{K}^+$  disequilibrium. On one hand, prokaryotic membranes became largely impermeable not only to sodium ions but also to protons, and the proton-dependent bioenergetics, which should have been more beneficial under oxidizing conditions [19], became prevalent. On the other hand, the ancestors of eukaryotes evolved a specialized, extremely efficient enzyme to maintain the  $\text{Na}^+/\text{K}^+$  disequilibrium, namely the  $\text{Na}^+/\text{K}^+$  ATPase, which couples hydrolysis of a single ATP molecule with the transport of 3  $\text{Na}^+$  ions out of the cell and 2  $\text{K}^+$  ions into the cell. As a result, in animal and plant cells, the major systems of  $\text{Na}^+/\text{K}^+$  exchange ( $\text{Na}^+/\text{K}^+$ -ATPase and ion channels) became uncoupled from the membrane energy-converting machinery (the  $\text{H}^+$ -translocating enzymes of endosymbiotically obtained mitochondria and chloroplasts).

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