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Packaging Life: The Origin of Ion-Selective Channels

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Most articles dealing with early life focus on its chemical basis and the evolution of proteins, RNA, DNA, and other metabolic products. This essay, however, is concerned primarily with the energy required to produce and maintain the essential life chemicals, and the necessity to confine them in a cell where they can function cooperatively.

It seems likely that life evolved in proximity to the underwater vents discovered by the submersible craft Alvin of the Woods Hole Oceanographic Institute (Woods Hole, MA). Bacteria were probably the original life form, growing in mats near the vents. Some advantages of the vents as starting points for life are:

1) Plentiful water and essential ions. We are ~60% salt water.
2) Plenty of chemical elements, many out of equilibrium and ready to combine. From one point of view, chemistry is simply the search of electrons, e.g., the two around a hydrogen molecule, for vacancies as close as possible to a nucleus with many protons, e.g., oxygen, or less avid electron gatherers, e.g., nitrogen, carbon, sulfur, or phosphorus. Eighteen of the 20 AAs contain only hydrogen, carbon, nitrogen, and oxygen; and the remaining two require sulfur in addition. DNA and RNA require, in addition, phosphorus. In short, it is not necessary to dip far into the periodic table to make most of the life chemicals.
3) Energy can be derived from combining chemicals issuing from the vent, as in a fuel cell, which derives energy by passing electrons from hydrogen to oxygen.
4) The water near a vent is warm, speeding experiments in the evolution of life chemicals.

Given these essentials, it is easy to imagine that life developed over a period of time, say a billion years. J. D. Sutherland, who first succeeded in synthesizing RNA, was quoted as saying: “My assumption is that we are here on this planet as a fundamental consequence of organic chemistry...So it must be chemistry that wants to work” (1). This essay starts at the point where early chemistry has done its work. It deals with the packaging of the life chemicals, the problems that arise from packaging, and energy production.

Packaging

How are the molecules of life held together? Clearly nature’s answer is to gather them within cells defined by a bounding bilipid membrane. The membrane, made of hydrophobic lipid, prevents ions and other hydrophilic material (i.e., most of the substances found dissolved in sea water) from entering the cell, unless there are specific channels or carriers for entry of desirable material. At the same time, a lipid membrane retains the large and generally hydrophilic life chemicals (proteins, phosphates, etc.). Although it is relatively impermeable to water, water nonetheless permeates a cell membrane rather quickly (equilibration in seconds to minutes; Finkelstein (2)) because of its very high molarity, ~54 M in sea water. The lipid membrane thus is not an effective barrier to water movement, creating a (very familiar) osmotic problem, as discussed next.

The osmotic problem

Suppose that a trial cell is surrounded by a bilipid membrane that has very low, nonselective permeability to all sea water ions, and low permeability to water. The trial cell is immersed in sea water (osmolality of 1 OsM), and internally contains sea water to which we add 1 OsM of life chemicals, making an initial internal osmolality of 2 OsM. The 1 OsM of life chemicals can be said to dilute the sea water inside the cell, making the water concentration (and the concentration of the sea water ions) lower than outside. Both water, at a relatively quick pace, and sea-water ions, at a relatively slow pace, will then diffuse into the cell to dilute the life chemicals. This causes the cell to swell, with two possible outcomes: 1) If the membrane does not resist, swelling will continue until the life substances are infinitely dilute (i.e., cell volume is infinite) and the membrane is infinite in area. 2) If the membrane strongly resists swelling, the intracellular pressure will rise and prevent water and sea-salt entry. How much pressure would be required? The answer is given by the Morse equation,

$$\Pi = iMRT.$$ 

In this equation, $\Pi$ is the osmotic pressure, i.e., the pressure (internal minus external) required to prevent water entry and
swelling; \( i \) is the dimensionless van’t Hoff factor, \(-1\) for a nonelectrolyte; \( M \) is the total molality of the cell contents; the product \( iM \) is the osmolality; \( R \) is the gas constant; and \( T \) is the absolute temperature. For 2 \( \text{OsM} \) internally and 1 \( \text{OsM} \) externally, the net osmotic pressure (at 25°C) is 22.4 atm, tending to expand the cell. A bilipid membrane is about as tenuous as a soap bubble, and could not withstand this pressure. Further, it would break after expanding only 4–5% (3). It is almost certainly for this reason that bacteria have a tough cell wall, which provides resistance to swelling and membrane rupture, and can withstand high pressure. In this regard, it helps that bacteria are very small. The Young-Laplace law states that for a spherical cell,

\[
\sigma = \Delta P r / 2,
\]

where \( \sigma \) is the membrane tension, \( \Delta P \) is the transmembrane pressure, and \( r \) is the radius. Thus a cell with small radius requires less membrane tension to withstand a given pressure.

We return to the osmotic question after considering the chemiosmotic mechanism for energy production invented long ago by bacteria, but most thoroughly examined in mitochondria. Chemiosmosis exacerbates the osmotic problem.

**Energy from chemiosmosis in mitochondria**

The earliest bacteria, e.g., the model cell in the preceding section, may have used a relatively simple system for energy production similar to glycolysis. But components of the more complex chemiosmotic mechanism, which might be called a biological fuel cell, are at least 1,500,000,000 years old (4). In a fuel cell, electrons fall down an energy gradient from hydrogen to oxygen, producing voltage, current, and water. The electrons are attracted from hydrogen to orbits around oxygen atoms that have more protons to attract the electrons, and vacancies in orbits relatively close to the attractive, proton-containing nucleus. One of the great insights of the last century was the chemiosmotic hypothesis of Peter Mitchell (10): mitochondria harness the energy of electrons passing from hydrogen to oxygen, in a scheme very analogous to a fuel cell.

In mitochondria (which derive from ancient bacteria), hydrogen and electrons are provided by the citric acid cycle in the form of NADH and FADH₂, both of which have loosely bound high-energy electrons.

Following the path of the NADH electrons (Fig. 1), they are presented to the first of a series of proton pumps, often referred to as the electron transport chain. The electrons surrender energy to each of the pumps as they move downhill energetically to the bottom of the energy hill, where they bind tightly to an oxygen atom. The energy received by each pump is used to move protons outward through the mitochondrion’s inner membrane. One result is that the pumps produce a pH difference between outside and inside of 1.4 pH units (25× higher proton concentration outside (4)). More important energetically, the export of positively charged protons leaves a deficit of positive charge inside, sufficient to create a membrane voltage \( (V_m) \) of \(-140 \text{ mV} \) negative inside. The combined voltage and proton concentration gradient (electrochemical gradient) is used to drive protons inward through ATP synthase, transforming ADP + Pᵢ into ATP, the gasoline of the cell. Using the numbers just given, \(-63% \) of the energy for driving ATP synthase is provided by the membrane voltage of \(-140 \text{ mV} \), which sucks protons into the mitochondrion. The remaining energy comes from the proton gradient.

**Osmotic considerations in mitochondria**

The mitochondrion is an intracellular organelle, bathed in cytoplasmic fluid that contains a high K⁺ concentration (~150 mM), very low Ca²⁺, and ~1 mM free Mg²⁺. Most of the anionic charge in the cytoplasm is on macromolecules and phosphates. The interior of the mitochondrion is negative to the cytoplasm, making it essential that the permeability to all cations be very low. From the Nernst equation in the form

\[
[X]_{\text{out}} / [X]_{\text{in}} = \exp(V_m Z F / RT),
\]

the equilibrium concentration of ions can be calculated. Here, \([X]_{\text{in}}\) and \([X]_{\text{out}}\) are the internal mitochondrial and cytoplasmic concentrations of an ion, \(V_m\) is the membrane voltage, \(Z\) is the given ion’s valence, \(F\) is the Faraday constant, \(R\) is the gas constant, and \(T\) is the absolute temperature. Free permeability to K⁺ with a membrane voltage of \(-140 \text{ mV} \) would result in an intramitochondrial K⁺ concentration of 32.3 M, clearly an impossibility: K⁺ permeability must be quite restricted, and there must be a pump using energy from ATP or the proton electrochemical gradient to export K⁺ and keep its
Chemiosmosis in a sea water bacterium

Chemiosmosis in bacteria is quite ancient, although, in early times, electron donors other than H₂ and electron acceptors other than O₂ were probably used. Cytochrome c₃, a key component of the electron transport chain, is >1,500,000,000 years old (4). Although chemiosmosis is much more efficient than glycolysis, the osmotic problem described above is aggravated by the negative membrane voltage necessary for energy production by chemiosmosis. Fig. 2 shows a hypothetical bacterium in sea water. The bacterium is assumed to have an internal voltage of 140 mV, the value required for producing ATP in mitochondria. Imagine for a moment a freely permeable membrane that allows free passage to all ions without discrimination. From the voltage and the sea-water ion concentrations shown, the intracellular concentrations of Na⁺, Ca²⁺, and Mg²⁺ are impossibly high: the bacterium would be a salt crystal long before these concentrations could be achieved. In short, the bacterium could not survive with a significant permeability to these ions. For K⁺, the intracellular concentration is high, but possibly in the range for a bacterium in sea water. Interestingly, the negative internal voltage drives Cl⁻ out of the hypothetical bacterium through a chloride (more accurately, anion) channel, lowering its internal concentration to 3 mM.

The italicized numbers in the figure show the concentrations for a bacterium that is impermeable to Na⁺, Ca²⁺, and Mg²⁺; has a highly selective K⁺ channel; and has a Cl⁻ channel. The K⁺ concentration inside at equilibrium would be ~2 M. There would be a low concentration of other cations (not shown) due to imperfect exclusion by the K⁺ channel and the bilipid membrane. The concentrations of the unwanted ions would be kept low by cation pumps that use energy from the proton gradient or ATP to pump the ions outward. Cl⁻ ion permeates through chloride channels, but thanks to the negative internal voltage, its concentration, in compliance with the Nernst equation, would be only 3 mM. The requirement for electroneutrality is satisfied by putting one or more negative charges on the molecules making up the life chemicals that are confined within the cell. By this means, electroneutrality is achieved (except, of course, for the tiny excess of negative ions that charge the membrane capacitance to ~140 mV). That is, internally the cell would have mainly K⁺ at a concentration of ~2 M, negatively charged life chemicals, and a small amount of HCO₃⁻ and Cl⁻. This arrangement would bring the cell sufficiently close to osmotic equilibrium such that its cell wall could withstand any remaining force of osmotic origin. As discussed below, gating of the K⁺ channel may also be important in limiting osmotic strain.

In summary, the presence in bacterial membrane of a highly selective K⁺ channel and a Cl⁻ channel in combination with the chemiosmotic machinery provides a means of efficient ATP production and ameliorates the osmotic problem, such that the cell wall can withstand any remaining osmotic pressure. Without a highly K⁺ selective channel and an anion channel, the chemiosmotic requirement for a large negative membrane voltage would result in an osmotic disaster. Chemiosmosis in sea-water bacteria could not have evolved without a K⁺ channel able to exclude Na⁺, Ca²⁺, and Mg²⁺, a chloride channel to allow equilibration of Cl⁻ at a low level internally, and pumps to counteract the leak of unwanted cations.

Channel gating in chemiosmotic bacteria

One can only speculate regarding the usefulness to a bacterium of the voltage-dependent K⁺ channel-gating, seen, for example, in bacterial KvAP channels (5), which close when the membrane voltage is negative to ~−50 mV. The combination of a K⁺ channel and a negative membrane voltage obviates the need for a pump to bring in K⁺, because the negative membrane voltage sucks in K⁺. However, a voltage of ~140 might well draw in more K⁺ than wanted, which would have to be pumped out. This would make it desirable to close the channel when the voltage is very negative, thus limiting excessive influx of K⁺ ion and the energetic cost of pumping it out. Hence, at least hypothetically, there would be need for a voltage-gated K⁺ channel.

Another type of gating, called C-type inactivation, would be useful should a bacterium (not confined to sea water) encounter a medium with little or no K⁺ outside. K⁺ channels endowed with C-type inactivation close (inactivate) in very low K⁺ medium, and would thus lower K⁺ loss (6,7).
Animal cells (Fig. 3) differ from bacteria in (at least) two important respects: 1) The task of ATP production has been taken over by the mitochondria, which are presumed to have developed from bacteria swallowed long ago by ancestral eukaryotic cells. 2) Animal cells are not surrounded by a cell wall. Like a bacterium, they have a negative internal membrane voltage, \(~-60\) to \(-90\) mV vs. \(~-140\) mV for a bacterium. If not for ATP production, what is the function of the membrane voltage in an animal cell? The first thought that comes to mind, of course, is electrical signaling as in nerve and heart cells. However, all animal cells have a negative internal membrane voltage, including those that have no electrical signaling function. Instead, then, the negative internal voltage is for solving the osmotic problem that animal cells face, caused by the life chemicals that are present only within the cell.

Our cells, like the primordial bacterial cells considered above, are bathed in a medium that is high in NaCl and low in K\(^+\)—essentially, diluted sea water. Why do animal cells go to the trouble of pumping Na\(^+\) out of and K\(^+\) into the cell, a task upon which the human brain uses \(-20\)% of the body’s basal oxygen consumption (8)? The reason for this was first clearly elucidated by Tosteson and Hoffman (9): it is necessary for osmotic balance. In a bacterium, the internal osmolality is acceptably low because of the low Cl\(^-\) and HCO\(_3^–\) concentrations inside the cell. Electro-neutrality is achieved thanks to negative charges on the life chemicals. In an animal cell, internal Cl\(^-\) is held low by the negative internal membrane voltage: at \(-70\) mV, Cl\(^-\) at equilibrium is \(14.7\times\) lower internally than externally. The membrane voltage in an animal cell thus arises not as a by-product of ATP production, but from a combination of the Na/K pump, which raises internal K\(^+\) to \(~150\) mM, and a K\(^+\)-selective membrane channel. The idea of using internal negativity to lower internal Cl\(^-\) and HCO\(_3^–\), making osmotic room for the life chemicals, was inherited from bacteria, and is so successful that nowhere in nature has an ATP-driven Cl\(^-\) pump been found: Cl\(^-\) concentration is controlled primarily by membrane voltage. Thanks to this osmotic solution, our cells are in osmotic equilibrium and the need for a cell wall is removed, not partially as in a bacterium, but completely. Were our cells encased in rigid walls as are plant cells, we would be, in the words of Tosteson and Hoffman, mute dryads of the trees.

The membrane voltage and the Na\(^+\) gradient are also used to transport substances into and out of the cell. An example is the mechanism developed to dispose of the CO\(_2\) produced by chemiosmosis in the mitochondria. The enzyme carbonic anhydrase converts CO\(_2\) into H\(_2\)CO\(_3^–\), which dissociates into HCO\(_3^–\) and a proton. HCO\(_3^–\) exits the cell via the Cl\(^-\) (anion) channel, and through a chloride bicarbonate exchanger. The proton is ejected from the cell by a Na\(^+\)-H\(^+\) exchanger. Both bicarbonate and proton ejection are dependent on energy from the Na\(^+\) electrochemical gradient.

The potassium channel is the most selective of all known channels with regard to discriminating between the ions in sea water or animal blood. This high degree of selectivity was first necessitated by its role in solving the osmotic problem in bacteria, and was later useful in setting the resting voltage of animal cells. Because the membrane is not completely impermeable to other cations, it is necessary, as in bacteria, to pump out the unwanted cations using an energy source, e.g., ATP, for the pump. The pumps are probably variants of pumps originally developed in bacteria.

The most important early use of membrane voltage was in energy production (chemiosmosis) and osmotic balance. Other uses, such as signaling, developed later. Once given the resting potential, only a Na\(^+\) channel was necessary to develop most of the electrical signaling that underlies sensory perception, long distance transmission to the central nervous system, and a major part of brain function. The eukaryotic Na\(^+\) channel and its cousin, the Ca\(^{2+}\) channel, apparently were made by stitching together the four subunits of a K\(^+\) channel into a single molecule with four domains and then differentiating the four domains to increase selectivity to Na\(^+\) (Na\(^+\)/K\(^+\) selectivity is \(~10\)) and to provide the complex inactivation-gating seen in Na\(^+\) channels. The chemically gated channels used to mediate communication among cells probably evolved from bacterial-membrane receptors that had employed them for chemotactic attraction to nutrients.

REFERENCES


