



Why Are Cells Powered by Proton Gradients?

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The proton gradients that power respiration are as universal as the genetic code itself, giving an insight into the origin of life and the singular origin of complexity

Why do virtually all cells "breathe" by pumping protons (hydrogen ions) across a membrane? According to molecular biologist Leslie Orgel, this is the single most counterintuitive idea in biology after Darwin's, and the only one to bear comparison with the concepts of Heisenberg, Schrödinger, and Einstein (Orgel 1999).

Pioneered by the eccentric British biochemist Peter Mitchell (Figure 1), largely in his own research laboratories in a renovated country house in rural Cornwall, the concept was controversial for more than twenty years. This period of controversy was known as the "ox-phos wars" (after "oxidative phosphorylation," the mechanism of ATP synthesis in respiration). The wars drew to an end only after Mitchell received the Nobel Prize in 1978.

There's an irony here. Mitchell's Nobel was for work in chemistry, yet his ideas are actually about the elimination of chemistry. In the same way that the genetic code enables information to transcend chemistry, so Mitchell's proton gradients enable cellular metabolism to transcend chemistry.

The use of proton gradients gives an insight not only into how life got going in the first place, but also, perhaps, its deepest stalling point: the evolution of complex eukaryotic (nucleated) cells, which arose just once in 4 billion years of evolution.

How Cells Breathe

Our own cells burn food with oxygen and contrive to conserve the energy released in the form of ATP (the universal energy currency of life) — a process called aerobic respiration. How do cells do it? More to the point, how don't they?

Back in the 1940s, Efraim Racker had just figured out the mechanism by which cells glean a little energy from the breakdown of glucose in the absence of oxygen, a pathway known as glycolysis. In glycolysis, phosphate groups are transferred directly from sugar molecules onto ADP to form ATP. The whole pathway is pure chemistry, involving the reaction of one molecule with another, and therefore obeys the laws of stoichiometry; that is, you can balance the equations. Not surprisingly, Racker and others immediately tried to transfer their insights to the quantitatively far more important process of aerobic respiration, which supplies more than 80 percent of our ATP.

But one glaring problem with aerobic respiration is that it doesn't balance. Exactly how much ATP is produced per oxygen molecule consumed?

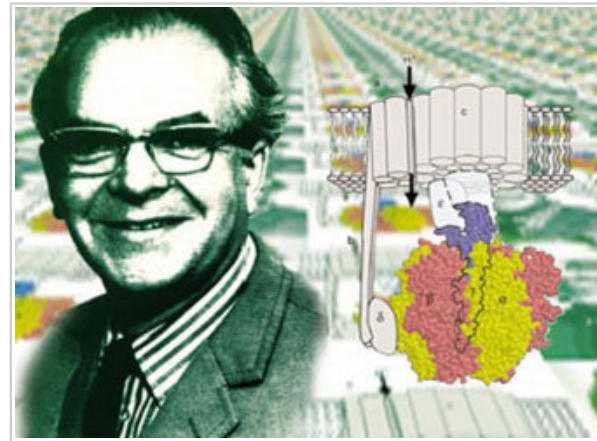



Figure 1: Peter Mitchell and the ATP synthase enzyme
© 1999 Nature Publishing Group Orgel, L. Are you serious, Dr Mitchell? *Nature* 402, 17 (1999). doi:10.1038/46903 All rights reserved. 

The amount varies, but it's somewhere around 2.5 ATP molecules. That works out to 28–38 ATPs per glucose — again, a **variable** number, and never an integer (Silverstein 2005). Aerobic respiration is not stoichiometric, so it's really not chemistry. And that's why the long search for a high-energy chemical intermediate (a molecule able to transfer the energy from the oxidation of glucose to form ATP) was doomed to failure.

In place of such an intermediate, Mitchell proposed a proton gradient across a membrane: the proton motive force (Mitchell 1961). It works much like a hydroelectric dam. The energy released by the oxidation of food (via a series of steps) is used to pump protons across a membrane — the dam — creating, in effect, a proton reservoir on one side of the membrane. The flow of protons through amazing **protein** turbines embedded in this membrane powers the synthesis of ATP in much the same way that the flow of water through mechanized turbines generates electricity. This explains why respiration is not stoichiometric: a gradient, by its very nature, is composed of gradations.

Fine Details

Mitchell was completely wrong about some of the details, but his overall conception was right, even revolutionary — literally revolutionary, as in, the ATP synthase **enzyme revolves**. The flow of protons through the membrane turbines rotates the stalk of the ATP synthase, and the conformational changes induced by this rotation catalyze ATP synthesis. This mechanism was first proposed by Paul Boyer, who long disagreed with Mitchell over the mechanics and was later proved right in this particular by John Walker, using **X-ray crystallography**. Boyer and Walker shared the Nobel Prize in 1997.

The molecular biological achievements of the last two decades culminated in 2010 with the deciphering of the crystal structure of another respiratory complex, the enormous (for a protein) complex I, by Walker's Cambridge colleague Leonid Sazanov (Efremov *et al.* 2010). Again, the structure betrays the mechanism — in this case not a rotary motor but, even more surprisingly, a lever mechanism not unlike the piston of a steam engine (Figure 2).

Yet without in any way decrying these virtuosic accomplishments, the questions that drove Mitchell in the first place remain surprisingly unanswered. We know in nearly atomic detail how respiration works. We know far less about why it works that way.

Proton Motivation

Mitchell worked on **mitochondria** because he could; they are a tractable experimental **model**. But he came at the question from the standpoint of bacterial physiology — how do bacteria keep their insides different from the outside? Throughout his life, Mitchell saw the detailed mechanism of respiration in this far broader sense: Membrane proteins can create gradients across a membrane, and these gradients can in turn power work. Proton gradients powering ATP synthesis were just a special case to Mitchell.

What he can hardly have envisaged so clearly was the pervasive role of protons. Although cells can generate sodium, potassium, or calcium gradients, proton gradients rule supreme. Protons power respiration not only in mitochondria, but also in **bacteria** and **archaea** (members of another **domain** of prokaryotes, which look much like bacteria but have very different biochemistry). Proton gradients are equally central to all forms of **photosynthesis**, as well as to bacterial motility (via the famous **flagellar motor**, a rotary motor similar to the ATP synthase) and **homeostasis** (the import and export of many molecules in and out of the **cell** is coupled directly to the proton gradient). Even fermenters, which don't need proton gradients to generate ATP, maintain the proton motive force, using ATP derived from fermentation to power proton pumping.

In short, Mitchell knew protons were important, but he could hardly have guessed at just how important. But why protons? Because they were there from the very beginning, says Michael Russell, a geochemist at NASA's Jet Propulsion Laboratory in Pasadena.

Proton Gradients at the Origin of Life

For the last two decades, Russell has been the dynamic force behind the emerging paradigm shift in our understanding of the **origin** of life. Drawing on a background in ore geochemistry (many ores are precipitated by

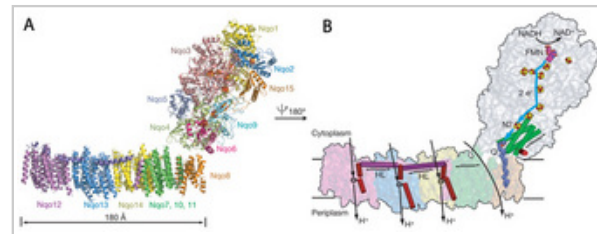


Figure 2: The structure of complex I, the largest protein complex involved in respiration in bacteria and mitochondria, as revealed by X-ray crystallography

The structure (a) suggests the piston mechanism shown (b), whereby shunting the piston drives protons across the membrane through three separate channels.

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hydrothermal vent systems), Russell postulates that alkaline vents, akin to the modern Lost City vent system in the mid-Atlantic (Figure 3), were the ideal incubators for life, providing a steady supply of hydrogen gas, carbon dioxide, mineral catalysts, and a labyrinth of interconnected micropores (natural compartments similar to cells, with filmlike membranes; Lane *et al.* 2010). Alkaline vents are, in essence, electrochemical reactors that operate in a state far from equilibrium. But the centerpiece of Russell's conception lies in natural proton gradients. Four billion years ago, alkaline fluids bubbled into what would then have been mildly acidic oceans (CO₂ levels were about a thousand times higher than they are today, and CO₂ forms carbonic acid in solution, rendering the oceans mildly acidic). Acidity is just a measure of proton concentration, which was about four orders of magnitude (four pH units) higher in the oceans than in vent fluids. That difference gave rise to a natural proton gradient across the vent membranes that had the same polarity (outside positive) and a similar electrochemical potential (about 200 millivolts [mV] across the membrane) as modern cells have.

Russell has long maintained that natural proton gradients played a central role in powering the origin of life. There are, of course, big open questions — not least, how the gradients might have been tapped by the earliest cells, which certainly lacked such sophisticated protein machinery as the ATP synthase. There are a few possible abiotic mechanisms, presently under scrutiny in Russell's lab and elsewhere. But thermodynamic arguments, remarkably, suggest that the only way life could have started at all is if it found a way to tap the proton gradients (Lane *et al.* 2010).

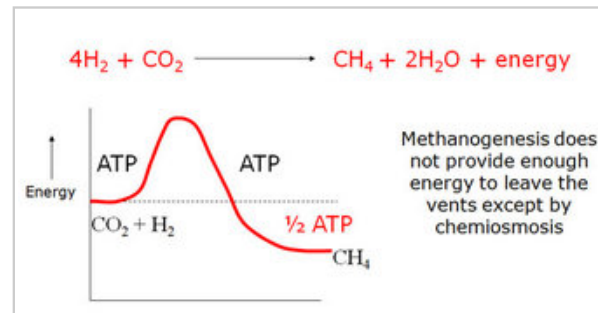
Why Proton Gradients Are Necessary


The reason proton gradients are required boils back down to chemistry. Life hydrogenates carbon dioxide. In other words, to convert carbon dioxide into organic molecules, life attaches hydrogen atoms to CO₂. There are only so many ways of doing this, and all life uses just five primary pathways. All but one of these costs energy (for example, the energy of the sun in photosynthesis). The exception is an ancient pathway called the acetyl-CoA pathway, in which hydrogen gas is reacted, via a few steps, with CO₂. This pathway is exothermic (releasing energy that can be captured as ATP) right through to pyruvate, one of the central molecules in cell metabolism. It's "a free lunch that you're paid to eat," in the words of Everett Shock.

But there's a problem, pointed out by William Martin in collaboration with Russell (Martin & Russell 2007). All cells that use the acetyl-CoA pathway today depend on proton gradients. None of them can grow by fermentation — that is, by the chemistry of glycolysis. Why not? Because CO₂ is a stable molecule and does not react easily, even with hydrogen — even when thermodynamics says it should react. CO₂ is a bit like oxygen in this respect: Once it starts to react, it's not easily stopped. But a fire needs a spark to get it going, and so, too, does CO₂. Cells need the equivalent of a spark to get CO₂ to react, and for cells, that spark is ATP. The problem is that the reaction of CO₂ with H₂ releases energy, but not a lot — only enough to make 1 ATP. That means cells have to spend 1 ATP to gain 1 ATP, so there's no net gain. If there's no gain, there's no growth; no growth, no life.

Gradients break that cycle. It's not quite true to say that the reaction of CO₂ with H₂ releases enough energy to make 1 ATP: it's actually enough to make 1.5 ATPs. But of course there's no such thing as 1.5 ATPs, at least not by stoichiometric chemistry; so the spare energy from the reaction, as chemistry, is lost (Figure 4). But that doesn't happen with a gradient (Figure 5). In principle, a reaction can be repeated over and over again, just to pump a proton over a membrane. When enough protons have accumulated, the proton motive force powers the formation of ATP. So a gradient allows cells to save up protons as "loose change", and that makes all the difference in the world — the difference between growth and no growth, life and no life.

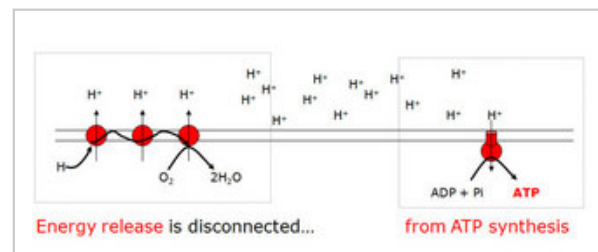
 **Figure 3**



 **Figure 4: Why chemistry is not enough**

Substrate level phosphorylation costs 1 ATP to gain 1ATP. Net growth is not possible.

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The Origin of Complex Life

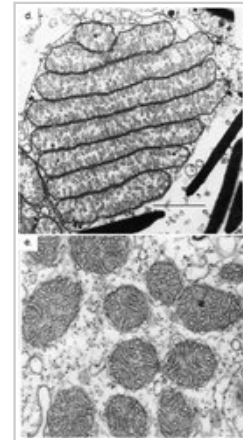
 **Figure 5: Why chemiosmosis solves the problem**

If a reaction doesn't release enough energy to generate 1 ATP, it can be repeated endlessly until it has pumped enough protons to generate 1 ATP. Chemiosmosis allows cells to save loose change, so to speak.

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Despite their power, protons have their share of problems, and these problems might explain why life got stuck in a rut for 2 billion years. All complex life on Earth today is composed of a certain type of complex cell, known as a eukaryotic cell. Generally much larger than bacteria or archaea, the eukaryotic cell contains a **nucleus**, and a much larger **genome**, and all kinds of specialized organelles (little organs), such as mitochondria. The strange thing is that eukaryotes have repeatedly given rise to large, complex, multicellular organisms like plants, animals, fungi, and algae — but prokaryotes show little or no tendency to evolve greater morphological complexity, despite their biochemical virtuosity. Why not?

One possible answer relates to the control of proton gradients. All eukaryotic cells turn out to have mitochondria, or once had them and later lost them by reductive evolution back toward a prokaryotic state. No mitochondria, no eukaryotes (Figure 6). All mitochondria capable of oxidative phosphorylation have retained a tiny genome of their own, which appears to be necessary to maintain control over membrane potential (Allen 2003). A membrane potential of 150 mV across the 5-nanometer membrane gives a field strength of 30 million volts per meter — equivalent to a bolt of lightning. This huge electrochemical potential makes the mitochondrial membranes totally different from any other membrane system in the cell (such as the endoplasmic reticulum) which, according to Allen, is why mitochondrial **genes** are needed locally in cellular subregions. In effect, by responding to local changes in electrochemical potential, they prevent the cell from electrocuting itself. No mitochondrial genome, no oxidative phosphorylation. It could be, then, that bacteria can't expand in cell and **genome size** because they can't physically associate the right set of genes with their energetic membranes. Lacking mitochondria, bacteria cannot grow large and complex, because they can't control respiration over a wide area of energetic membranes (Lane & Martin, *in press*). If that's the case, the acquisition of mitochondria and the origin of complexity could be one and the same event.



 **Figure 6**

The question is, what kind of a cell acquired mitochondria in the first place? Most large-scale genomic studies suggest that the answer is an archaeon — that is, a prokaryotic cell that is in most respects like a bacterium. That begs the question, how did mitochondria get inside an archaeon? The answer is a mystery but might go some way toward explaining why complex life derives from a single common ancestor, which arose just once in the 4 billion years of life on Earth.

Summary

Peter Mitchell's demonstration that ATP synthesis is powered by proton gradients was one of the most counterintuitive discoveries in biology, and it took a long time to be accepted. The precise mechanisms by which a proton gradient is formed and coupled to ATP synthesis (chemiosmotic coupling) is now known in atomic detail, but the broader question that drove Mitchell — why are proton gradients so central to life? — is still little explored. Recent research suggests that proton gradients are strictly necessary to the origin of life and highlights the geological setting in which natural gradients form across membranes, in much the same way as they do in cells. But the dependence of life on proton gradients might also have prevented the evolution of life beyond the prokaryotic level of complexity, until the unique chimeric origin of the eukaryotic cell overcame this obstacle.

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