PRESIDENTIAL ADDRESS

Molecular Machines and Ion Propulsion: A Random Walk By a Mitochondriac

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It has been quite some time now since I gave my Presidential Address to the Academy, and now I am faced with the task of putting it in printed form. The project of translation from my oral presentation to a written account has me somewhat stymied. In my talk, I relied heavily on slides as something I could talk around, a format I am familiar with as a teacher and researcher. Now I have to put it in bare words without the benefit of illustrative materials that make speaking so much easier. Also, the particular slides have long since been refiled various places, and I am uncertain what some of them were. So be it.

My talk was based partly on my direct research experiences and partly on related biological phenomena that I just find interesting but have not personally studied in the laboratory. One of my research interests involved developing methods to measure pH within various cellular compartments. The measurement of pH within cells and subcellular compartments had become interesting when it was realized that cells have active mechanisms to regulate internal pH, and that each subcellular compartment seems to have its own system to do so. Also, it had finally been accepted, due largely to the singular persistent voice of one scientist working alone on his estate (Peter Mitchell), that transmembrane electrochemical gradients of protons (hereafter called "proton gradients" for simplicity) are the driving force for ATP formation in mitochondria, bacteria, and chloroplasts, as well as for other biological energy conversion processes, including the transport of nutrients and locomotion in bacteria.

I focused my talk on two systems that convert transmembrane proton gradients into other useful energy forms. One system was the mitochondrial ATP synthase, which converts the energy inherent in the proton gradient into ATP (chemical energy). The other system involved the flagella of bacteria, which use proton gradients to propel the bacterium (mechanical energy). In both systems, the movement of protons through a specific membrane assembly apparently drives the rotation of a molecular motor. A crude analogy would be the wind (the proton gradient) striking the blades of a windmill (our molecular motor), causing them to turn and do useful work. Structurally speaking, this rotor-stator analogy is not bad for either system. The rotational aspect is well established for the bacterial system and somewhat less so for the mitochondrial ATP synthase.

Flagella propel a bacterium forward by rotation in a counter-clockwise direction. When the motor rotation is in this direction, all the individual flagella cooperate by fitting together in a single large flagellar bundle that drives the bacterium ahead in a straight line. However, periodically the molecular motor momentarily reverses to a clockwise direction. The individual flagella cannot form a bundle when rotating clockwise. This causes the flagellar bundle to fly apart and the bacterium to tumble. After tumbling briefly, the motor quickly re-establishes its original counter-clockwise direction, the flagellar bundle reforms, and the bacterium proceeds to travel off in a new direction. Thus bacterial motion involves a series of straight line movements interrupted by tumbling,which causes it to set out in a new direction. Of course, it is the proton gradient that drives the motor in both clockwise and counter-clockwise directions. The details of how this reversible motor actually functions are still a subject of active investigation.

Since the tumbling process is random, so is the new direction. So how does the bacterium get anywhere? Without any regulation, this would simply result in a random walk. The bacterium accomplishes net movement by adjusting the length of time it moves ahead before reversing the motor and tumbling. If the direction is favorable (i.e., towards food, light, etc.), the motor reversal occurs less frequently. If the new direction is unfavorable, it undergoes tumbling much sooner. Hence, movement towards a desired goal is essentially a process of trial and error. It seems to change direction for variety (change for change's sake) but soon realizes its mistake if that change is unfavorable, and tries a different direction.

The other system I discussed was the ATP synthase, an enzyme which is found in bacteria, plants, and animals. In all systems studied, the structure of the enzyme is essentially conserved. Although the energy used to create the proton gradients varies from system to system (e.g., light in plants, oxidative processes in animals), the basic mechanism for ATP synthesis is the same in all three systems. The three-dimensional structure of this enzyme is known. Basically, it consists of a three-bladed turbine-like structure (headpiece) that sits atop a shaft that extends into the membrane. The contacts between this shaft and the turbine blades are mostly hydrophobic; that is to say, they are "well oiled." It is believed that the movement of the stream of protons through the membrane causes the shaft to rotate with respect to the turbine.

So how is the rotation of this shaft coupled to ATP formation? The ATP synthase can, in fact, make a limited amount of ATP in the absence of a proton gradient. If one adds a molar equivalent of ADP and inorganic phosphate to the enzyme, one finds approximately a mole of ATP is formed, but that it is tightly bound to the enzyme. In fact, it is this binding energy that pulls this otherwise unfavorable reaction towards completion.

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\begin{array}{lll}
(1) & \text{ADP + Pi} & \overset{\rightharpoonup}{\longleftharpoondown} \quad \text{ATP} + \mathrm{H_2 0} \\
(2) & \text{ATP + ENZYME} & \overset{\longrightarrow}{\leftharpoondown} \quad \text{[ENZYME-ATP]} \\
\hline
\text{NET} & \text{ADP + Pi + ENZYME} & \overset{\longrightarrow}{\leftharpoondown} \quad \text{[ENZYME-ATP]}
\end{array}
$$

Unfortunately, because the ATP is so tightly bound to the enzyme, it is unavailable for use. The role of the proton gradient is to release it from the enzyme. It does this by causing rotation of the asymmetric shaft. Because the shaft is asymmetric ("wobbles"), rotation essentially causes it to "knock" the bound ATP off a subunit as it contacts it. This is a rather unsophisticated description and an oversimplification, to which my mitochondrial colleagues would probably take offense, but I think it provides a useful visual image of what occurs. Besides, a little inaccuracy saves tons of explanation. Because of the asymmetric nature of the shaft, each of the three identical turbine parts (subunits) of the headpiece is in a slightly different conformation at a given time. In other words, interactions with the shaft forces each to assume a slightly different three-dimensional shape. Thus, at a given time, one subunit is poised to bind ADP and phosphate, another to bind ATP very tightly, and the third is unable to bind nucleotides at all. As the shaft rotates, each subunit in turn assumes the shape best suited to its interaction with the shaft, with the structural changes occurring in the order as described. Thus, a given subunit first binds ADP and phosphate, then assumes a shape that favors it converting the ADP and phosphate to tightly bound ATP, and finally that ATP is released as it is forced into the third conformation. The process keeps being repeated as the shaft rotates, with each subunit undergoing these transitions sequentially, but 120 degrees out of phase with each other.

I punctuated my talk with aphorisms or "take-home lessons" that were related or could be gained from the science that was discussed. It was sort of the researcher's version of *All I Really Need To Know I Learned in Kindergarten,* a book that has been popular recently. Actually my inspiration for this approach was a pair of books on bioenergetics written by Efraim Racker [1,2], one of the pioneers in characterizing the components of the mitochondrial ATP synthesizing enzyme. In those books he accented his scientific narrative with witticisms from various historical sources. In fact, a number of the sayings I used in my talk were lifted directly from the books of Racker, who, of course, had collected them from various sources. I conclude this account with a listing of some of those sayings but, unlike in my actual talk, without any attempt to integrate them into the narrative.

FRUSTRATIONS AND FAILURES

"Troubles are good for you."

"It doesn't matter if you fall down, as long as you pick up something from the floor while you get up."—Efraim Racker [1]

"A man learns to skate by staggering about making a fool of himself. Indeed, he progresses in all things by resolutely making a fool of himself."—George Bernard Shaw, in *Advice to a Young Critic*

NEW THEORIES AND SCIENTIFIC PROGRESS

"Progress is made by young scientists who carry out experiments old scientists said wouldn't work."—F. Westheimer

"Listening to both sides of a story will convince you that there is more to a story than both sides."—Frank Tyger

"It takes time for an old theory to be replaced by a new one, even if it is simpler and more encompassing. The reason for this long time lag was explained by Max Planck. He remarked, 'Scientists never change their minds, but they eventually die.' "—E. Racker [1]

"Even if you are on the right track, you'll get run over if you just sit there."—Will Rogers

"When the dust settles, thou will see whether thou ridest a horse or an ass."—Chinese Proverb [1]

"It is the customary fate of new truths to begin as heresies, and end as superstitions."—Thomas Henry Huxley

"The difficulty lies not in new ideas, but in escaping the old ones."—J. M. Keynes

"All general statements are false." (Including this one.)—Unknown

"Things are only impossible until they are not."—Jean-Luc Pickard

"Discovery consists of seeing what everybody has seen and thinking what nobody has thought."—Albert von Szefft-Gyorgi

"An undefined problem has an infinite number of solutions."—Robert A. Humphrey

THE NATURE OF SCIENTISTS

"If you can't do it spectroscopically, it's not worth doing."—Britton Chance

"Scientists have odious manners, except when you prop up their theory; then you can borrow money from them."—Mark Twain

"Only two things are infinite, the universe and human stupidity, and I'm not sure about the former."—Albert Einstein

REFERENCES

A New Look at Mechanisms in Bioenergetics. Efraim Racker. Academic Press, Inc., 1976.

Reconstitutions of Transporters, Receptors, and Pathological States. Efraim Racker. Academic Press, Inc., 1985.