The Biochemistry of Life's Energy

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Abstract: On December 10, 1997, the 101st anniversary of Alfred Nobel's death, in Stockholm's Concert Hall Swedish King Carl XVI Gustav awarded one half of the 1997 Nobel Prize in Chemistry (3.75 million kronor, about $500,000) to Professor Emeritus Paul Delos Boyer, age 79, of the University of California, Los Angeles and Dr. John Ernest Walker, age 56, of the Medical Research Council Laboratory of Molecular Biology, Cambridge, England "for their elucidation of the enzymatic mechanism underlying the synthesis of adenosine triphosphate (ATP)" and one half (3.75 million kronor, about $500,000) to Professor Emeritus Jens Christian Skou, age 79, of Århus University, Århus, Denmark "for the first discovery of an ion-transporting enzyme, Na^+, K^+-ATPase."

Bioenergetics, that branch of biochemistry dealing with energy transformations in living organisms, plays a key role in the chemistry of human life, and the award of the 1997 Prize for Chemistry, that *ne plus ultra of science*, to three researchers working independently in three different countries on enzymes that store and transfer energy in the body's cells follows the award of the prize to earlier bioenergeticists such as Peter D. Mitchell in 1978 and Hartmut Michel in 1988.

According to the Royal Swedish Academy of Sciences:

> The three laureates have performed pioneering work on enzymes that participate in the formation and use of the "high energy" compound adenosine triphosphate (ATP).... [Their] work demonstrates how the body's cells capture and use energy, fundamental processes that affect everything from the building of bones to the contraction of muscles and the transmission of nerve impulses.

Boyer and Walker showed how the enzyme ATP synthase catalyzes the formation of ATP. On the basis of biochemical data Boyer and his co-workers proposed a mechanism for how ATP is formed from adenosine diphosphate (ADP) and inorganic phosphate. Walker and his co-workers established the structure of the enzyme and verified the mechanism proposed by Boyer. Skou and his co-workers discovered the enzyme that maintains the critical balance of sodium and potassium ions in the living cell. Both enzymes are bound to membranes in the cell and are linked with the transport of ions through these membranes, but for different reasons.

Paul Delos Boyer

Boyer (Figure 1 and 2), one of six children of Dell Delos Boyer, an osteopathic physician, and Grace Boyer (née Guymon), was born on July 31, 1918 in Provo, Utah where he attended primary (in which he skipped a grade) and secondary schools. His father's forebears came from Pennsylvania and were descended from the Bayers of Holland and Germany. His mother's ancestors were Huguenots who had fled religious persecution in France. More by example than by word, his father "taught him logical reasoning, compassion, love of others, honesty, and discipline applied with understanding." When he was fifteen, his mother died at the early age of 45 of Addison's disease, and her death, which he maintains could have been prevented by later discoveries about adrenal hormones, contributed to his subsequent interest in biochemistry. He admits having a bad temper, which perhaps "was later sublimated into drive and tenacity, traits that may have come from [his] mother." His first exposure to chemistry resulted from a chemistry set that he received as a Christmas present. In high school where he was class valedictorian, he participated in intramural basketball, debating, and student government (He was senior class president).

Boyer attended Brigham Young University in Provo, only a few blocks from his home, where he concentrated on chemistry and mathematics; a painstaking course in qualitative and quantitative analysis "gave [him] an appreciation of the need for, and beauty of, accurate measurement." He worked summers as a waiter and managerial assistant at an inn near Salt Lake City. As a member of the Medical Corps of the National Guard, he spent several weeks in a military camp in California. During his senior year he considered several career choices—a chemist in the mining industry, hotel management, osteopathic or conventional medicine, or some type of graduate training. "Having a tendency to be lucky and make the right choices based on limited information," he applied and was approved for a Wisconsin Alumni Research Foundation (WARF) scholarship for graduate study. Boyer received his B.S. degree in 1939, the year in which he married Lyda Whicker on August 31, by whom he later had two daughters (Gail and Hali) and a son (Douglas). His wife later became a professional editor at the University of California, Los Angeles and worked with him on the 18-volume series, *The Enzymes* (1970–90).

The Biochemistry Department at the University of Wisconsin, Madison was outstanding and far ahead of most others in the country (Brigham Young University did not include biochemistry in its curriculum). Among its distinguished faculty Harry Steenbock had just patented the irradiation of milk for enrichment with vitamin D, Conrad A. Elvehjem's group had discovered that nicotinic acid would cure pellagra, William H. Peterson's group was identifying and separating bacterial growth factors, and Karl Paul Link's group was isolating and identifying a vitamin K antagonist from

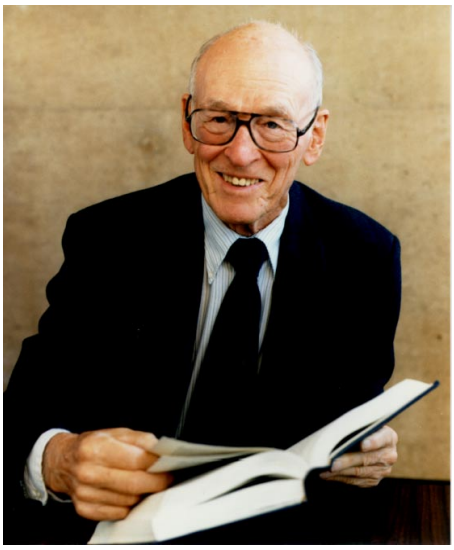

Figure 1. Paul Delos Boyer, © The Nobel Foundation, 1998.

Figure 2. Paul Delos Boyer holding his nobel prize medal with wife Lyda (Photo couresy of Dr. Paul D. Boyer).

sweet clover. A new wing in the biochemistry building had recently been opened, and the excitement of vitamins, nutrition, and metabolism permeated the department. In Boyer's words, "This superb training environment set the base for my career."

Supported by his scholarship and his wife's earnings, Boyer pursued graduate studies at Madison under Professor Paul Phillips with whom he explored metabolism and enzymes, interests that were emphasized by the younger faculty. A few months after his arrival in Madison he suffered an attack of appendicitis; "through medical mismanagement" his appendix ruptured and he became deathly ill, but sulfanilamides, discovered only a few years earlier by Gerhard Domagk (1939 Nobel laureate in Physiology or Medicine), saved his life. At an evening seminar Boyer presented evidence (obtained with fellow graduate student Henry Lardy) for the first known K^+ activation of an enzyme, pyruvate kinase. He received his M.S. and Ph.D. degrees in 1941 and 1943, respectively.

Boyer became an instructor and research associate at Stanford University (1943–45), where he participated in a Committee on Medical Research wartime project that resulted in a still-used stabilization method for concentrated serum albumin fractionated from blood plasma and gave him "experience with proteins and a growing respect for the beauty of their structures." After a year in the U.S. Navy at the Navy Medical Research Institute in Bethesda, Maryland, he became assistant professor at the University of Minnesota where he rose through the ranks to professor (1946–55) and Hill research professor at the Medical School (1955–63) and where he continued his work on metabolism, enzyme action, and protein structure and function and where he struggled "with the interpretation of some puzzling isotope exchanges accompanying an enzyme catalysis." Although he and his students spent much time on enzymes other than ATP synthase, "solving how oxidative phosphorylation occurred remained one of the most challenging problems of biochemistry." In addition to exchange reactions involving ^{18}O, he and his co-workers spent several years trying to detect a possible phosphorylated intermediate in ATP synthesis using ^{32}P as a probe. The work culminated in the discovery of a new type of phosphorylated protein, a catalytic intermediate in ATP formation with a phosphoryl group attached to a histidine residue. Boyer spent his sabbatical year 1955–56 in Sweden as a Guggenheim Fellow working at the Wenner-Gren Institute of the University of Stockholm with Olov Lindberg and Lars Ernster and at the Nobel Medical Institute with (Axel) Hugo Theorell, who received the Nobel Prize in Physiology or Medicine that very year (1955).

In the summer of 1963 Boyer and his students and postdocs then moved to the University of California, Los Angeles where he was professor of chemistry (1963–89) and director of the newly founded Molecular Biology Institute (1965–83). Here he found that the newly discovered enzyme-bound phosphohistidine was an intermediate in the substrate level phosphorylation of the citric acid cycle rather than a key to oxidative phosphorylation, a finding that reminded him of a favorite saying, "Most of the yield from research efforts comes from the coal that is mined while looking for diamonds."

Boyer has been chairman, Biochemistry Study Section of the U.S. Public Health Service (1962–67); chairman, American Chemical Society Division of Biological Chemistry (1959–60); member, U.S. National Committee for Biochemistry (1965–71) and National Academy of Sciences (1970); president, American Society of Biological Chemists (1969–70); and fellow, American Academy of Arts and Sciences. He was editor of *Annual Review of Biochemistry* (1965–70), *Biochemical and Biophysical Research Communications* (1969–80), and *The Enzymes* (1970–90) and editorial board member of *Biochemistry* (1969–75). Among his honors and awards are the ACS Award in Enzyme Chemistry (1955), the Rose Award of the American Society for Biochemistry and Molecular Biology (1989), the 12th annual Glenn T. Seaborg Medal (1998), and honorary doctorates from Stockholm University (1974), the University of Minnesota (1996), and the University of Wisconsin (1998). He became Professor Emeritus at UCLA in 1989 but is still active as a scientist.

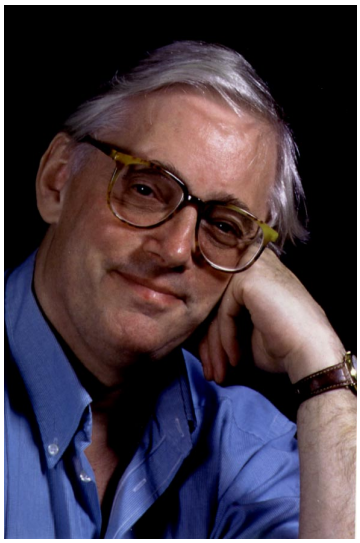

Figure 3. John Ernest Walker, © The Nobel Foundation, 1998.

A man of strong moral convictions, Boyer has raised a number of provocative and thought-provoking issues in the concluding paragraphs of his Nobel biographical sketch:

> The study of life processes has given me a deep appreciation for the marvel of the living cell. The beauty, the design, and the controls honed by years of evolution, and the ability humans have to gain more and more understanding of life, the earth, and the universe are wonderful to contemplate. I firmly believe that our present and future knowledge of all that we are and what surrounds us depends on the tools and approaches of science. I was struck by how well Harold Kroto, one of last year's Nobelists, presented what are some of my views in his biographical sketch. As he stated, "I am a devout atheist—nothing else makes sense to me, and I must admit to being bewildered by those, who in the face of what appears so obvious, still believe in a mystical creator." I wonder if in the United States we will ever reach the day when the man-made concept of a God will not appear on our money, and for political survival must be invoked by those who seek to represent us in our democracy.
>
> It is disappointing how little the understanding that science provides seems to have permeated into society as a whole. All too common attitudes and approaches seem to have progressed little since the days of Galileo. Religious fundamentalists successfully oppose the teaching of evolution, and by this decry the teaching of critical thinking. We humans have a remarkable ability to blind ourselves to unpleasant facts. This applies not only to mystical and religious beliefs, but also to long-term environmental consequences of our actions. If we fail to teach our children the skills they need to think clearly, they will march behind whatever guru wears the shiniest cloak. Our political processes and a host of human interactions are undermined because many have not learned how to gain a sound understanding of what they encounter.
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> The major problem facing humanity is that of the survival of ourselves and our progeny....Humans could become quite transient occupants of planet Earth. The most important cause of our problem is overpopulation, which nature, as with other species, will deal with severely. I hear the cry from capable environmental leaders and organizations for movement toward sustainable societies....But their voices remain largely unheard as those with power, and those misled by religious or national concerns, become immersed in unimportant, self-centered, and short-range pursuits.

John Ernest Walker

Walker (Figure 3), the youngest of the three laureates, was born on January 7, 1941, the eldest of the three children (he had two sisters—Judith and Jennifer) of Thomas Ernest Walker, a stone mason and amateur pianist and vocalist, and Elsie Walker (née Lawton), in Halifax, England. He grew up in a rural environment and was educated at Raistrick Grammar School (1953–60) where he was school captain in soccer and cricket and specialized in physical sciences and mathematics. He attended St. Catherine's College, University of Oxford (1960–64) from which he received his B.A. degree in chemistry. He then carried out research on peptide antiobiotics with Edward Penley Abraham at the Sir William Dunn School of Pathology, University of Oxford from which he received his M.A. and D. Phil. degrees, both in 1969. He married Christina Westcott in 1963; the couple have two daughters, Esther and Miriam, who are presently studying geography and English, respectively, at Nottingham-Trent and Leeds universities.

John Kendrew's series of programs on BBC television, which were published in 1966 as *The Thread of Life*, acquainted Walker with the spectacular developments made in molecular biology at Cambridge University during the 1950s and 1960s and inspired him to learn more about the subject. Two books, James D. Watson's *Molecular Biology of the Gene* (1965) and William Hayes's *The Genetics of Bacteria and Their Viruses* (1964), helped to assuage his appetite for more information, and two exciting series of lectures, one by David Phillips, Oxford's new professor of molecular biophysics, and another by Henry Harris, Oxford's professor of pathology, published as *Nucleus and Cytoplasm* (1974), influenced him greatly.

Walker then spent five years working abroad as a postdoctoral fellow at the School of Pharmacy, University of Wisconsin, Madison (1969–71) and as a NATO fellow at the Centre National de la Recherche Scientifique (CNRS), Gif-sur-Yvette (1971–72) and a European Molecular Biology Organization (EMBO) fellow at the Institut Pasteur, Paris (1972–74), both in France.

Just before Easter in 1974 Walker attended an EMBO research workshop on "Sequence Analysis of Proteins" organized by Ieuan Harris of the Medical Research Council's Laboratory of Molecular Biology (LMB) at Cambridge University and Richard Perham of Cambridge's Department of Biochemistry. At the symposium banquet Walker found himself seated next to 1958 Nobel chemistry laureate Frederick Sanger, who asked him if he had thought of returning to work in England. Walker approached Harris about the possibility of joining his group, and after discussions with Sanger he agreed to became a scientific staff member of the Division of Protein and Nucleic Acid Chemistry (PNAC) Division of LMB for three months (June–August, 1974). He still works at LMB today and states that "this encounter with Fred Sanger and Ieuan Harris transformed my scientific career."

In 1974 the three divisions of the LMB were the site of revolutionary discoveries in molecular biology that inspired Walker to make his own contributions. Along the corridor

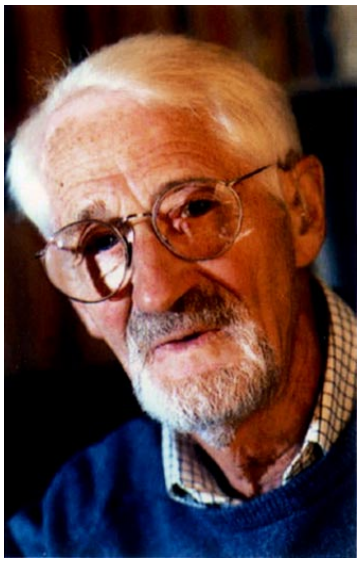

Figure 4. Jens Christian Skou, © The Nobel Foundation, 1998.

from Walker's laboratory Sanger was inventing his methods for sequencing DNA, immediately across the corridor Georges J. F. Köhler and César Milstein were developing monoclonal antibodies, and in the same building Francis H. C. Crick and Aaron Klug were revealing the structures of chromatin and transfer RNA. In the half century of its existence nine MRC-LMB staff members have received ten Nobel prizes—Chemistry: Sanger (1958, before he joined the LMB; 1980), John C. Kendrew and Max F. Perutz (1962), Klug (1982), and Walker (1997); Physiology or Medicine: Crick and Watson (1962, for work done at LMB after they had left) and Köhler and Milstein (1984).

Sanger's new sequencing methods were applied first to the related bacteriophages ϕX174 and G4 and then to DNA from human and bovine mitochondria. Walker analyzed the sequences of the proteins from G4 and from mitochondria using direct methods. This work led to the discovery of triple overlapping genes in G4 (with D. Shaw and B. G. Barrell) and to the discovery that subunits I and II of cytochrome c oxidase are encoded in the DNA in mitochondria. He also helped to uncover the modified genetic code in mitochondria. At the MRC-LMB he served as senior scientist (1982–87), and he now holds a special (professorial grade) appointment (1987–).

The author of about 200 articles, Walker has been an editorial board member of *The Biochemical Journal* (1981–87), *Biochemistry International* (now *Biochemistry and Molecular Biology International*, *BAMBI*) (1987–), and *Molecular Microbiology* (1987–91); and he is an editorial board member of *Journal of Bioenergetics* (1991–) and *Structure* (1993–); deputy editor of *DNA Sequence* (1989–); a member of the Scientific Advisory Board of the E. C. Slater Institute, University of Amsterdam (1989–) and The Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen (1994–), both in The Netherlands; an honorary member of the Department of Biochemistry, University of Oxford (1997–); and member of the Molecular and Cellular Medicine Board of the Medical Research Council, UK (1997–).

Walker's honors and awards include the A. T. Clay Gold Medal for academic distinction (1959); the University of Pennsylvania's Johnson Foundation Prize (1994); Smith-Kline Beecham Visiting Professorship to the U.S.A., Royal Society of Medicine Foundation (1995); the CIBA Medal and Prize, Biochemical Society (1995); the Peter Mitchell Medal, European Bioenergetics Congress (1996); and the Gaetano Quagliariello Prize for Mitochondrial Research, University of Bari, Italy. A fellow of the Royal Society since 1995, in 1997 he became a fellow of Sidney Sussex College, Cambridge and honorary fellow of St. Catherine's College, Oxford.

Walker's research on mitochondria and their genetic codes led to his work on enzyme complexes in the inner membrane of the organelles that carry out oxidative phosphorylation. His structural study of the ATP synthase from bovine heart mitochondria, begun in 1978, eventually resulted in a complete sequence analysis of the complex and in an atomic resolution structure of the F_1 catalytic domain of the enzyme. In keeping with the rotatory mechanism of ATP synthase, Walker stated, "I was thinking I'd buy myself a new bicycle" with his Nobel Prize winnings.

Jens Christian Skou

Skou (Figure 4), the oldest of four children (he had two brothers and one sister) of Magnus Martinus Skou, a wealthy timber and coal merchant, and Ane-Margrethe Knak Skou (née Jensen), was born on October 8, 1918 in Lemvig, a small town near the North Sea in western Denmark. When he was twelve, his father died, and his paternal uncle continued the business so that there was no change in the family's economic situation. Because there was no boarding school (*gymnasium*) in Lemvig, beginning at the age of fifteen, he spent the last three years of school in Haslev on the island of Zealand, where his favorite subjects were science and mathematics, but where there was plenty of time for sports and scouting. In 1937 he entered the University of Copenhagen to study medicine for seven years—three years of chemistry, physics, anatomy, biochemistry, and physiology and four years of clinical subjects, pathology, forensic medicine, pharmacology, and epidemiology. In 1944, during the German occupation, he received his M.D. (*cand. med.*) degree.

Skou spent his years of clinical training (1944–47) at the hospital in Hjørring in northern Denmark and the Orthopædic Clinic at Århus. While at Hjørring he met Ellen Margrethe Nielsen, who became a nurse in 1948, the year that they married. After the birth in 1950 of a daughter, who died at the age of one, the couple had two more daughters—Hanne and Karen, born in 1952 and 1954, respectively. After the children left home, one to study medicine and the other to study architecture, Skou's wife worked for several years in a psychiatric hospital for children and then entered politics. Elected to the City Council as a Social Democrat, she served for twelve years on the council, first working on health care problems. She was also elected to the county Scientific Ethical Committee, which evaluates all research involving human beings, and then to the cochairmanship of the national Central Scientific Committee, an appeals committee for local committees and physicians. During her seventeen years on these committees, she has lectured to doctors and nurses on ethical problems.

In 1947 Skou joined the faculty of the Institute of Physiology at the University of Århus where he remained for more than four decades until his official "retirement" (he continues to pursue his research interests)—as assistant

professor (1947–54), associate professor (1954–63), professor and chairman (1963–78), and professor of biophysics (1978–88). Because his initial salary at the university was very low and because he wished to practice his profession as a doctor who saw patients, in 1949 he took an extra job as a physician on call one night a week. Although he had been born into a *milieu* that was politically conservative, his position as a doctor on call changed him into a Social Democrat who realized the importance of free medical care; free education with equal opportunities; and a welfare system that cares for the weak, the handicapped, the old, and the unemployed even if it results in higher taxes. A devoted husband and father, he restricted his work at the institute to a concentrated eight hours per day and spent the rest of his time, including weekends, holidays, and summer vacations with his family.

In 1954 Skou received his Doctor of Medical Sciences (*dr. med.*) degree from the University of Århus with a thesis on the action of local anesthetics that was published as a book in Danish and as six articles in English. This work led him to the identification of the sodium-potassium pump, which is responsible for the active transport of sodium and potassium ions across cell membranes (1957) and which gave him access to and contact with the outside scientific world. From this time his scientific interest shifted from the effect of local anesthetics to the active transport of cations.

The author of almost a hundred papers and reviews, Skou is a member of the Danish Academy of Sciences and numerous other scientific societies; a foreign associate of the U.S. National Academy of Sciences; and an honorary member of the Japanese Biochemical Society, American Physiological Society, and the New York Academy of Sciences. His awards and honors include the Leo Prize (1959), Novo Prize (1964), Consul Carlsen Prize (1973), Anders Retzius Gold Medal (1977), Eric K. Fernström's Nordic Prize (1985), and an honorary doctor of medicine degree from the University of Copenhagen (1986). Among his hobbies are yachting; skiing; reading, especially of biographies; and listening to classical music.

ATP, the Universal Energy Carrier of the Living Cell

Adenosine triphosphate (ATP) is a power-packed molecule described by Boyer as "the currency of the cell...the machine that makes the money that the rest of the body spends...[and without which] there would be no life at all." It was discovered in 1929 by German chemist Karl Lohmann. During 1939–41, the 1953 Nobel laureate in Physiology or Medicine Fritz A. Lipmann showed it to be the universal carrier of chemical energy in the cell of all living organisms from bacteria and fungi to plants and animals, including humans. He coined the expression "energy-rich phosphate bonds." It was synthesized in 1948 by 1957 Nobel Chemistry laureate Baron Alexander R. Todd.

ATP consists of the nucleoside adenosine linked to three phosphate groups. Removing the outermost phosphate group yields adenosine diphosphate, ADP, and releases energy that can be used for other biochemical processes requiring energy, such as the building of cell components, contraction of muscles, and transmission of nerve impulses. The reaction is reversible; with addition of energy, an inorganic phosphate group can be bonded to ADP to form ATP. By these reactions considerable amounts of ATP are produced and consumed; for example, at rest an adult human in a single day converts about half his or her body weight in ATP, while during hard work this amount can increase to almost a ton. In the human body most of the ATP synthesis is carried out by F_0F_1ATPase, now called ATP synthase, the enzyme first isolated in 1960 by Efraim Racker and later investigated by Boyer and Walker; while at rest about one third of all ATP formed is used up by Na^+, K^+-ATPase, the enzyme investigated by Skou.

Boyer's Work on Oxidative Phosphorylation

A modest man quick to give credit to others, Boyer began his Nobel address, "Energy, Life, and ATP," with the statement, "It is my good fortune to be a spokesman for a considerable number of outstanding researchers in the field of bioenergetics whose efforts have revealed an unusual and novel mechanism for one of nature's most important enzymes." This enzyme, ATP synthase, present in intracellular membranes of animal mitochondria, plant chloroplasts, bacteria, and other organisms, catalyzes oxidative phosphorylation, the vital cellular process by which our bodies derive from food the energy that we need for a myriad of essential cellular functions. According to Boyer, ATP synthase is "a remarkable molecular machine [which] accomplishes the oxidative phosphorylation that was left unexplained for over half a century." During the 1970s Boyer and his research group helped to overcome the limitations of older hypotheses for this process that were no longer applicable. They attempted to replace them with new, unexpected, and controversial hypotheses, for which they obtained additional experimental support during the 1980s and early 1990s. The most novel and least accepted aspect of these hypotheses was then confirmed by John Walker and co-workers' x-ray structural data on the catalytic portion of the enzyme that regulates phosphorylation.

Boyer estimates that

> The net synthesis of ATP is the most prevalent chemical reaction that occurs in your body. Indeed, because plants and microorganisms capture and use energy by the same reaction, and the amount of biomass is large, the formation and use of ATP is the principal net chemical reaction occurring in the whole world.

In animal mitochondria, enzymes, in a series of small steps, catalyze the oxidation of food, using oxygen from the air that we breathe and producing carbon dioxide. These series of oxidations liberate protons (hydrogen ions) and promote a charge that tends to force these protons across the membrane. In plant chloroplasts light energy is coupled to the formation of this protonmotive force. In 1961 Nobel laureate Peter Mitchell showed that this force causes protons to be translocated through the ATP synthase accompanied by formation of ATP, but just how the ATP synthase uses the protonmotive force to make ATP was an unanswered question for many years.

By the 1970s it was known that ATP synthase consists of a trio of protein assemblies: (1) a wheel-like structure imbedded in the membrane of the mitochondria; (2) a rod with one end attached to the hub of the wheel; and (3) a large cylinder that wraps around the rod's other end and adheres to the mitochondria's internal region. A number of researchers later demonstrated that ATP is synthesized at three sites on the

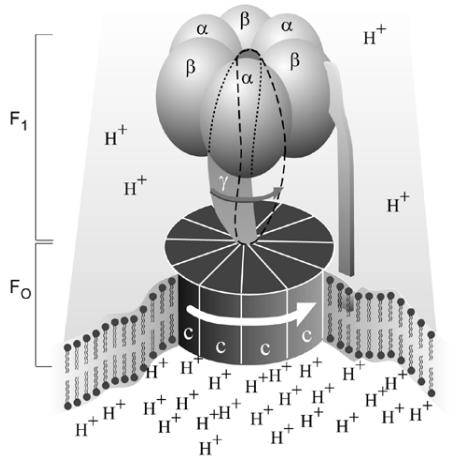

Figure 5. Simplified DIAGRAM OF ATP SYNTHASE, news release, the royal swedish academy of sciences, 1997.

The F_0 part, through which protons (H^+) stream, is located in the membrane, whereas the F_1 part, which synthesizes ATP, is outside the membrane. When protons flow through the membrane via the disc of c subunits in the F_0 part, the disc is forced to twist. The γ subunit in the F_1 part is attached to the disc and consequently rotates with it; however, the three α and three β subunits in the F_1 part cannot rotate because they are locked in a fixed position by the β subunit, which, in turn, is anchored in the membrane. Therefore, the γ subunit rotates inside the cylinder formed by the six α and β subunits. Because the γ subunit is asymmetrical, it forces the β subunits to undergo structural changes, leading to the β subunits binding ATP and ADP with differing strengths (See Figure 6).

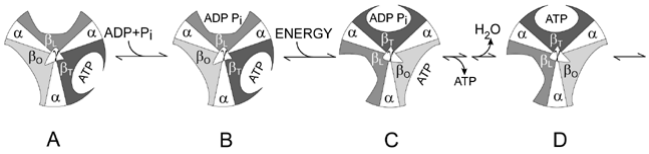

Figure 6. Boyer's "Binding Change Mechanism," news release, the royal swedish academy of sciences, 1997.

This diagram depicts the cylinder with alternating α and β subunits at four different stages of ATP synthesis (A through D). The asymmetrical γ subunit, which causes changes in the structure of the β subunits, is shown in the center. The structures are termed open β_o (light gray), loose β_L (gray), and dense β_T (black). At stage A an already fully formed ATP molecule is bound to β_T. In the step to stage B, β_L binds ADP and inorganic phosphate (P_I). At stage C the γ subunit has twisted due to the flow of protons (H^+) (See Figure 5), which causes changes in the structure of the three β subunits. The dense β subunit now becomes loose, and the bound ATP molecule is released. The loose β subunit becomes loose, and open β subunit becomes loose. In stage D the chemical reaction, in which phosphate ions react with the ADP molecule to form a new ATP molecule, occurs, returning the system to the original first stage.

cylinder and that the rod plays a central role in catalyzing this synthesis, but the mechanism remained unknown (Figure 5).

By the use of contributions of earlier workers and his own experiments involving ^{18}O, Boyer and co-workers proposed a novel mechanism consisting of catalytic steps different from any that had been observed previously with other enzymes. During net ATP synthesis, the three catalytic sites on the enzyme act in sequence; they first bind ADP and phosphate, change conformation to make a tightly bound ATP, and then change conformation again to release this ATP. He suggested that protons crossing the mitochrondrial membrane back to the central region cause the wheel-like structure to spin, much as rushing water turns a water wheel. The rod, which is attached to the wheel, also spins, causing the other end to rotate within the stationary cylinder. This rotation modifies the structure of the three catalytic sites within the cylinder, causing each of them in turn to snag the ATP's building blocks, to synthesize ATP, and to release it. Boyer's hypothesis incorporated three unusual features not previously recognized in enzymology: (1) energy-linked binding changes including release of a strongly bound ATP; (2) sequential conformational changes of three catalytic sites to accomplish these binding changes; and (3) a rotary mechanism that drives the conformational changes (Figure 6).

Walker's Work on the Structure of ATP Synthase

Because a detailed knowledge of the chemical and structural properties of an enzyme is needed to understand how it functions, during the 1980s John E. Walker began to study ATP. His work complements and confirms Boyer's work. He first determined the amino acid sequences in its constituent protein units, and during the 1990s he collaborated with X-ray crystallographers, the Dutchman Jan Pieter Abrahams and the Englishman Andrew G. W. Leslie, to elucidate the three-dimensional structure of ATP synthase obtained from bovine heart mitochondria. Their work reached a climax in August, 1994 when they determined the structure of the F_1 portion of the enzyme, the catalytic part consisting of the cylinder and rod. At the time it was the largest asymmetrical structure to be determined by x-ray crystallography. From this finding Walker went on to verify the rotating mechanism of the enzyme proposed by Boyer. Although it has been difficult to demonstrate experimentally, several groups in several countries have succeeded in doing this by different techniques, for instance, Wolfgang Junge by spectroscopy (Germany), Richard Cross by chemical cross-bonding (U.S.A.), and Masasuke Yoshida by microscopy (Japan).

Skou's Work on the First Discovered Molecular Pump

As early as the 1920s it was known that the ionic composition within the living cell differs from that outside the cell. In particular, the Na^+ concentration is lower and the K^+ concentration is higher than in the extracellular liquid. During the early 1950s two English scientists, Richard Keynes and 1963 Nobel laureate in physiology and medicine Sir Alan L. Hodgkin, showed that stimulation of a nerve is accompanied by a flow of Na^+ ions into the nerve cell. This difference in concentration is restored by the retransport of Na^+ out of the cell. Because this transport was observed to be inhibited by inhibiting the formation of ATP, it was likely that the transport required ATP.

At this time Skou was working on local anesthetics, and he thought that the effect of their penetration into the lipid portion of the nerve membrane was a blocking of the conformational change in proteins, which resulted in the increase in permeability to Na^+ ions. In 1948 Benjamin Libet had found an ATP-hydrolyzing enzyme in the sheath portion of the giant axon from squid. Inasmuch as ATP is the energy source in cells, Skou wondered what could be the function of an ATPase in the membrane of a nerve. Having no access to giant axons in Århus, he began to search for this nerve membrane ATPase

in crab nerves, which, like those of the giant axon, has no myelin sheath.

Skou's search in finely ground crab nerve membrane for an ATP-degrading enzyme associated with ionic transport resulted in his first article on an ATPase that was activated or stimulated by Na^+ and K^+ ions—Na^+, K^+-ATPase, the first enzyme that can promote directed transport of substances through a cell membrane, a fundamental property of all living cells. It was published in 1957 in *Biochimica et Biophysica Acta* under the title "The Influence of Some Cations on an Adenosine Triphosphatase from Peripheral Nerves," because Skou considered the word "pump" too provocative. "No wonder that few people noticed that this enzyme had to do with active transport of Na^+," related Skou. He further stated,

> Looking back, it was a very simple experiment to identify the pump. Just break the membrane and by this gain access to the Na^+ site on the inside and the K^+ site on the outside, add some ATP and test for the combined effect of Na^+ and K^+. It ought to have been done by someone who worked in the transport field and knew about active transport. I felt like an intruder in a field that was not mine.

Skou found that Na^+, K^+-ATPase requires the presence of Mg^{2+} ions in order to function, that Na^+ and K^+ ions bind with high affinity to different places on the enzyme, and that the phosphate group separated from ATP also binds (phosphorylates) to ATPase. Robert Wayne Albers and Robert L. Post, working independently of each other, showed that the enzyme depends on Na^+ ions on phosphorylation and on K^+ ions on dephosphorylation. In the years following the identification of this so-called Albers-Post scheme (Na^+-dependent phosphorylation, followed by the K^+-dependent dephosphorylation) a model for the transport reaction was developed by various workers.

By means of the gradients for Na^+ into the cell and K^+ out of the cell sustained by the pump, the cell solves an osmotic problem caused by the presence in the cell of negatively-charged protein molecules, which cannot penetrate the membrane. The gradients for the cations represent an energy source used for creation of an electrical potential across the cell membrane, which is the basis for the function of all excitable tissues, such as brain, nerves, and muscles. Furthermore, in the cell membrane there are transport molecules that use the gradients for the cations as an energy source for transport of other substances into and out of the cell against their electrochemical gradients and for transepithelial transport in the intestine, secretory glands, and kidney. The Na^+, K^+-ATPase thus acts as an energy transducer converting chemical energy from the hydrolysis of ATP into another energy source, the gradients for the cations.

Following Skou's discovery, about fifty other so-called ion pumps with similar structures and functions were discovered. For example, Ca^+-ATPase participates in the control of muscle contraction. H^+, K^+-ATPase, an enzyme that is inhibited in the modern treatment of stomach ulcers, produces hydrochloric acid in the stomach. Such enzymes are also found in lower organisms; for instance, an H^+-ATPase in yeast secretes H^+ ions formed during fermentation. Because these enzymes are phosphorylated during the course of their biochemical reactions, they are now commonly termed P-type ATPases.

The model for the transport reaction is a working hypothesis that explains many of the experimental observations, and Skou admits that there are some observations which do not agree with the model as well as many unanswered questions, especially at the molecular level. Skou concludes his Nobel address, "The Identification of the Sodium-Potassium Pump":

> It may seem disappointing that 30 years of work, since the conclusion that the membrane-bound Na^+, K^+-ATPase is identical with the Na^+, K^+-pump, has not given us an understanding of the basic molecular events behind the transport. However, considering that the problem is to reveal how 1320 amino acids inside a volume of 60 × 60 × 100 $Å^3$ can be assembled to a very efficient machine, which can convert the chemical energy from the hydrolysis of ATP into work, namely the transport of cations against their electrochemical gradient, and which can distinguish between such closely related cations as Na^+ and K^+, it can be no surprise that progress is slow. Thirty years ago it seemed impossible to find a way to purify the enzyme not to speak of getting it into solution and reconstitute it into liposomes. Nobody dared to dream about knowing the sequence, and yet this and much much more have been accomplished.

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