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Transport ATPases: Structure, Motors, Mechanism and Medicine: A Brief Overview

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Today we know there are four different types of ATPases that operate within biological membranes with the purpose of moving many different types of ions or molecules across these membranes. Some of these ions or molecules are transported into cells, some out of cells, and some in or out of organelles within cells. These ATPases span the biological world from bacteria to eukaryotic cells and have become most simply and commonly known as "transport ATPases." The price that each cell type pays for transport work is counted in molecules of hydrolyzed ATP, a metabolic currency that is itself regenerated by a transport ATPase working in reverse, i.e., the ATP synthase. Four major classes of transport ATPases, the P, V, F, and ABC types are now known. In addition to being involved in many different types of biological/physiological processes, mutations in these proteins also account for a large number of diseases. The purpose of this introductory article to a mini-review series on transport ATPases is to provide the reader with a very brief and focused look at this important area of research that has an interesting history and bears significance to cell physiology, biochemistry, immunology, nanotechnology, and medicine, including drug discovery. The latter involves potential applications to a whole host of diseases ranging from cancer to those that affect bones (osteoporosis), ears (hearing), eyes (macromolecular degeneration), the heart (hypercholesterolemia/cardiac arrest,), immune system (immune deficiency disease), kidney (nephrotoxicity), lungs (cystic fibrosis), pancreas (diabetes and cystic fibrosis), skin (Darier disease), and stomach (ulcers).

KEY WORDS: Transport ATPase; P-type ATPase; F-type ATPase; V-type ATPase; ABC transporters; cancer; cancer therapy; heart disease; cystic fibrosis.

DISCOVERIES

In the year 1957, the same year that the author of this article graduated from high school, one of his grandparent's Danish countrymen, Jens Skou (Fig. 1(A), left), would report experimental data demonstrating the existence of the first transport ATPase, i.e., the Na^+, K^+ ATPase, while working at the University of Aarhus in Denmark (Skou, 1957). In today's language of the National Institutes of Health and large medical schools like those of which the author is a member, Jens Skou would be considered a "physician scientist" as he was a physician (surgeon) trying to understand how anesthetics work by carrying out basic research. As an experimental system he chose both practically and wisely by selecting the shore crab as such were plentiful near the sea located close to the University of Aarhus, and shore crabs contained leg nerves of sufficient size to be subjected to a study of the effect of anesthetics thereon. For his discovery of the Na^+, K^+ ATPase, Skou won half the Nobel Prize in Chemistry in 1997, 40 years after reporting his discovery. In fact, one of his last reviews on the subject (Skou and Esmann, 1992) was published in this journal, the Journal of Bioenergetics and Biomembranes.

Crossing research paths with Skou was Robert Post (Fig. 1(A), right), a graduate of Harvard Medical School, and like Skou had a strong desire to understand basic mechanisms in physiology. In 1954, Post turned his attention to understanding sodium and potassium transport in erythrocytes, a system he also chose both practically

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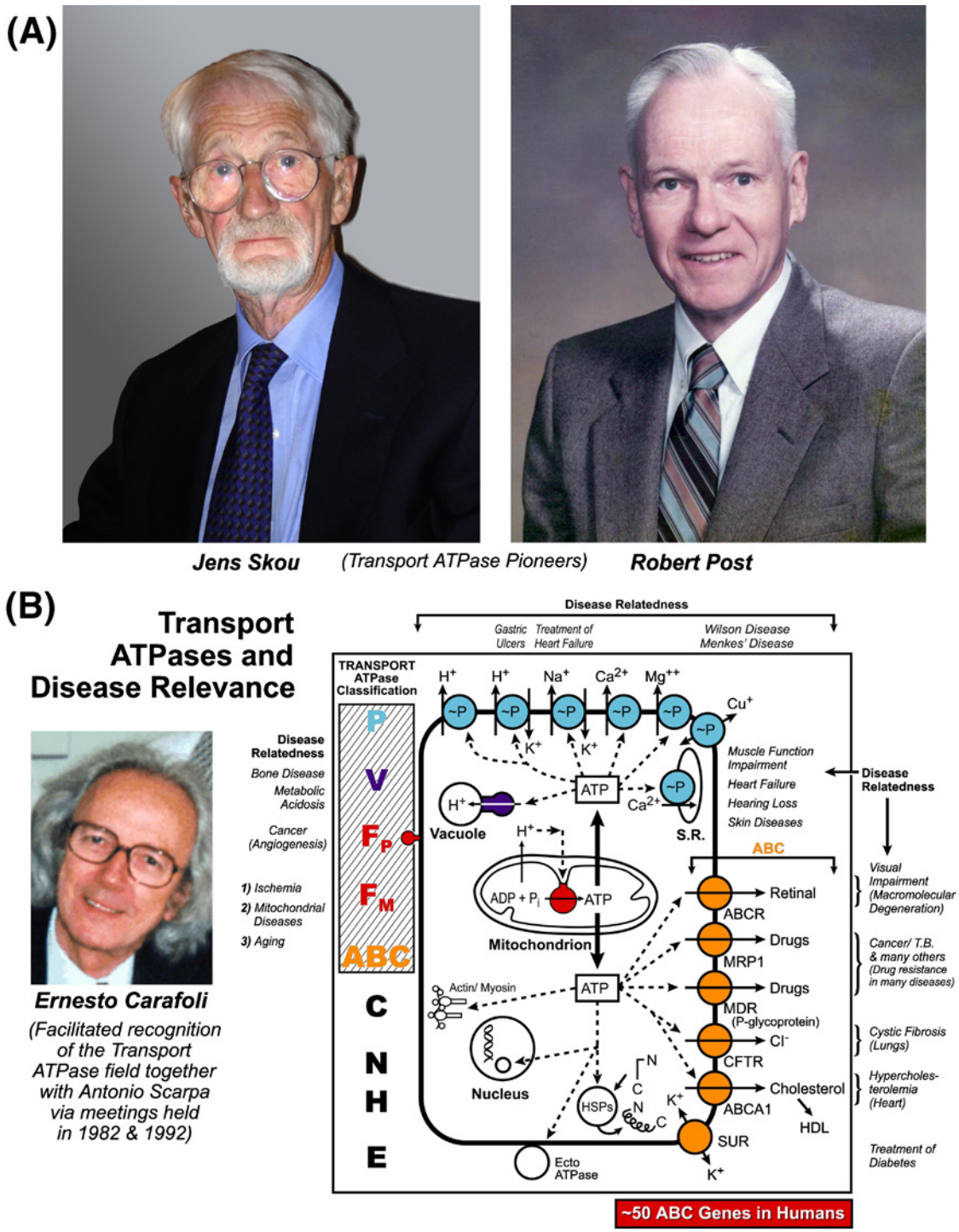

Fig. 1. (A) Transport ATPase Pioneers, Jens Skou (*left*) and Robert Post (*right*) played key roles in the development of the field focused on transport ATPases. Skou discovered the Na^+/K^+ ATPase and Post *et al*. helped elucidate its reaction pathway by showing that a key intermediate is a phosphorylated enzyme. These pioneering studies led to the discovery by others of ATPases with phosphorylated intermediates that have now become known as the P-Type. (B) Ernesto Carafoli (*left*), in addition to spending more than four decades working on calcium-related processes, has played a major role in organizing transport ATPase meetings. He has helped also name and categorize the various transport ATPases (*right*). Through his pioneering efforts together with those of Antonio Scarpa, the FASEB transport ATPase meetings were established. (The figure at right is a modified version of that published earlier by the author in this journal in 2002.)

and wisely as erythrocytes were metabolically simple and readily available, sometimes from Post's own blood. The same year Skou discovered the Na^+, K^+ ATPase in crab nerves, Post and Jolly (1957) at Vanderbilt University in Nashville Tennessee demonstrated in erythrocytes an electrogenic transport of 3 Na^+ ions outward per 2 Na^+ ions inward for that component of transport inhibited by a cardiac steroid. Several years later, Post and colleagues (Charnock *et al*., 1963; Post *et al*., 1965) would show that in the course of its ATPase reaction the Na^+, K^+ ATPase is covalently phosphorylated, a property unique to this class of transport ATPases now known as the "P-type" (Pedersen and Carafoli, 1987a,b) to designate their phosphorylation status at one point during the catalytic cycle.

In 1958, shortly following the 1957 discovery of the first P-type ATPase by Jens Skou, another scientist, Efraim Racker of the Public Health Research Institute in the city of New York had just completed together with colleagues Maynard Pullman and Harvey Penefsky some of their earliest work on mitochondria. Together they would report that a soluble ATPase from bovine heart mitochondria had the capacity to couple oxidation reactions (electron transport) to phosphorylation (ATP synthesis from ADP and P_i) in submitochondrial fragments (Pullman *et al*., 1958). The soluble ATPase was named F_1 and constitutes the catalytic headpiece of the enzyme now referred to as ATP synthase (F_0F_1) that is found in bacteria, plant chloroplasts, and mitochondria. As isolated, ATP synthase complexes catalyze ATPase activity as there is no driving force to reverse the reaction and make ATP. Such complexes are now referred to simply as "F-type" ATPases (Pedersen and Carafoli, 1987a,b) with the understanding that they can work in both directions.

Following the discovery of the F-Type ATPases, it would be almost a quarter century later when Yoshinori Ohsumi and Yasuihiro Anraku working at the University of Tokyo in Japan would report in 1981 that vacuoles of the yeast *Saccharmoyces cerevisiae* have the capacity to take up the amino acid arginine in an ATP hydrolysis dependent manner (Ohsumi and Anraku, 1981). This finding, together with the report of Emma J. and Barry J. Bowman of the University of California at Santa Cruz that vacuolar membranes of *Neurospora crassa* also contain a vacuolar ATPase (Bowman and Bowman, 1982) would nail down a third class of ATPases now referred to simply as "V-type" (Pedersen and Carafoli, 1987a,b).

The fourth and by far the largest class of transport ATPases are the "ABC-Type" (Pedersen, 2002), first recognized as belonging to a large superfamily by Christopher Higgins *et al*. (1986), then at the University of Dundee in Scotland. They are more commonly called "ABC transporters" where ABC specifies "ATP binding cassette," a term used by Hyde *et al*. (1990) and Higgins, 1992 in reference to the distinguishing feature of this transport ATPase class. Such transporters frequently contain the Walker A and B consensus sequences twice on the same polypeptide chain implicating two nucleotide binding sites, although the two nucleotide binding sites may be found also on separate polypeptide chains. Although such transporters were beginning to be isolated and studied nearly 20 years earlier (Ames and Roth, 1968), it was too early to recognize that they belonged to a very large superfamily. Today nearly 50 ABC-type ATPases (ABC transporters) are known to exist in humans (Dean and Annilo, 2005).

MEETINGS

When one looks back on the early history of the first ATPase types, i.e., P, V, and F, it is interesting to note that the discovery of each class was not made by research groups working near one another or even with ready access, e.g,. by phone, but rather by three different researchers (or groups) working in three different laboratories located on 3 different continents (Europe, North America, and Asia) and speaking three different languages (Danish, English, and Japanese). Such is the beauty of science as such discoveries have themselves generated extensive communications that have now brought together under one roof on at least seven different occasions scientists working not only on the P, V, and F-type ATPases but most recently also on the ABC-type ATPases. One of the first such meetings in the United States was organized in 1982 via the New York Academy of Sciences by Ernesto Carafoli (Fig. 1(B), left) then at the Swiss Federal Institute of Technology (ETH), Zurich, Switzerland and Antonio Scarpa, then at the University of Pennsylvania, Philadephia, USA. Five years after this meeting the author of this introduction and Ernesto Carafoli, in an attempt to further consolidate the transport ATPase field, would via two different articles (Pedersen and Carafoli, 1987a,b) subdivide the known transport ATPases at the time into the P, V, and F categories with the ABC type being included only recently (Pedersen, 2002). After a second transport ATPase meeting supported by the New York Academy of Sciences in 1992, an application was made to the Federation of American Societies for Experimental Biology (FASEB) for a full meeting sponsorship every 2 years. This was approved and highly interactive meetings have been held every 2 years since 1997 with respective chairs as follows: Antonio Scarpa in 1997, Ernesto

Carafoli in 1999, Peter L. Pedersen in 2001, Giuseppe Inesi in 2003, and Alan Senior in 2005, with the 2007 and 2009 Chairs respectively to be, Kathleen Sweadner and Rajini Rao.

DISEASE RELEVANCE

Figure 1(B), right is an attempt by the author to consolidate into a single figure the cellular locations of the transport ATPases, their primary transport functions, and their disease relevance. Such disease relevance may relate either to the treatment of the disease or to the cause of the disease, and includes many different diseases some of which are as follows: cancer, heart disease (including hypercholesterolemia), cystic fibrosis, diabetes, gastric ulcers, macromolecular degeneration, nephrotoxicity, osteoporosis, and deafness. Unfortunately, the figure does not do justice to many other diseases that result from mutations in ABC-type ATPases (ABC transporters) ranging from immune deficiency to sideroblastic anemia and ataxia (Gottesman and Ambudkar, 2001; Dean and Annilo, 2005). However, it will be pointed out that one of the hottest areas of research on ABC transporters in recent years relates to the ABC transporter referred to as ABCA1 or ABC1 that is involved in the reverse cholesterol transfer pathway involving HDL. As defects in this ABC transporter can result in hypercholesterolemia and heart disease, it has taken on added interest both for the purpose of better understanding the reverse cholesterol transport pathway and for developing new therapies to modulate it (Brewer *et al*., 2004; Lee and Parks, 2005). In addition, the ABC transporters ABCG5, ABCG8, and ABCB4 (MDR2) are receiving considerable attention also as the major pathway for eliminating cholesterol from the human body is via secretion into the bile and these transporters facilitate this process (Yu *et al*., 2005; Langheim *et al*., 2005).

STRUCTURAL/MECHANISTIC ADVANCES

In terms of subunit types, the F and related V-type ATPases are much more complex than the P and ABC-type ATPases. For example, most of the enzymes in the latter two classes, with the Na^+, K^+ ATPase an exception, contain only a single polypeptide chain whereas the complete ATP synthase/ATPase complex in animal systems excluding regulators contains 15 subunit types with the catalytic F_1 unit alone containing five subunit types. The vacuolar ATPase is almost as complex. Therefore, one might have predicted that the P and ABC type ATPases would have been the first to be crystallized and solved structurally at atomic resolution via X-ray crystallography. However, as fate would have it, the more complex F-type ATPase (catalytic F_1 unit) with a subunit composition/stoichiometry of $\alpha_3\beta_3\gamma\delta\varepsilon$ Catterall *et al*., 1973 was the first to result in crystals suitable for X-ray analysis. Although two different groups of investigators would lead the way via a long and rocky path with intermittent disputes that entertained a decade of bioenergeticists at Gordon conferences, atomic resolution structures were eventually obtained.

F-Type ATPases

F_1 crystals (Fig. 2(A), left center) were obtained in diffraction quality form for the first time at Johns Hopkins University, School of Medicine (Amzel and Pedersen, 1978) with the author providing less than 5 mg F_1 to Amzel (Fig. 2(A), upper left) who crystallized it on the first try. Four years later subsequent work on additional preparations of these crystals would yield a 9 Å structure (Amzel *et al.,* 1982), the same year that Paul Boyer and colleagues (Gresser *et al*., 1982) proposed an alternating three-site model (Fig. 2(A), bottom left) for the synthesis of ATP by the F_1 catalytic unit of ATP synthase. Significantly, this model implied that one or more of the small subunits γ, δ, and ε may be moving. Nine years later a 3.6 Å structure of rat liver F_1 (not presented in Fig. 2) showed that the three α and three β subunits, rather than each sticking together, alternate within the structure (Bianchet *et al.,* 1991). Shortly thereafter the research group led by John Walker, Medical Research Council, Laboratory of Molecular Biology, Cambridge, UK, working with crystals of bovine heart F_1 obtained a 2.8 Å structure (Abrahams *et al*., 1994) This structure, the first atomic resolution structure of F_1, showed clearly that the γ subunit is centrally located running from near the top of F_1 to below its bottom, a finding that would be confirmed 4 years later in an atomic resolution structure (Fig. 2(A), left center) of rat liver F_1 (Bianchet *et al*., 1998). Finally, an improved structure by Walker and colleagues (Gibbons *et al*., 2000) would localize the other two small subunits (δ, and ε) to the foot of the γ subunit (Fig. 2, right center).

Although the centrally located γ subunit found in the 1994 atomic resolution structure of bovine heart F_1 was not completely visualized until the year 2000, the new information from the 1994 structure that the γ subunit runs through the center of F_1 suggested that this subunit might be the rotor of an ATP hydrolysis driven motor that

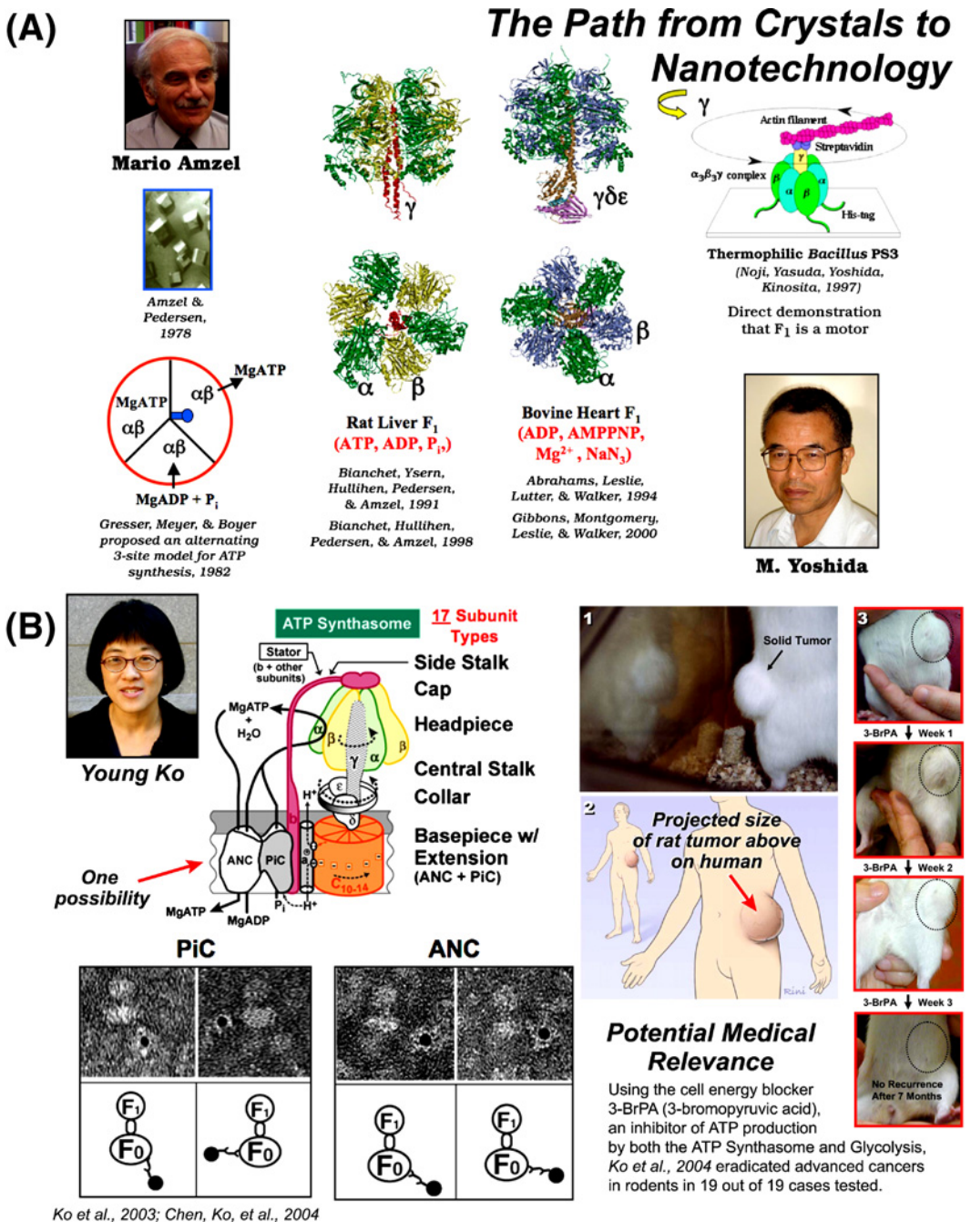

Fig. 2. (A) Summary of some of the key events starting from the first F1 crystals that eventually led to direct evidence that the F_1 catalytic unit of the ATP synthase is a nanomotor. This finding by Noji *et al*. (1997) gave strong support for the view that ATP synthesis from ADP and P_i by F_1 involves "rotary catalysis" as proposed earlier by Gresser *et al*. (1982). The key clue came from the 1994 crystal structure of Walker and colleagues that showed clearly that the single γ subunit runs through the center of F_1. This finding strongly implicated the γ subunit as the rotor of a motor. Putting this suggestion to the test, Yoshida *et al*. (1997) demonstrated directly the motor function of F_1. (B) Illustration that a challenging path lies ahead to fully understand the mechanism and regulation of ATP synthesis in mammalian mitochondria. (*left*) Evidence has been presented recently in work spearheaded by Young Ko (Ko *et al*., 2003; Chen *et al*., 2004) that in mammalian mitochondria the ATP synthase, excluding regulators, consists of at least 17 subunit types, two of which are accounted for by the phosphate carrier (PIC) and the adenine nucleotide carrier (ANC) that associate with the ATP synthase basepiece (see antibody binding lower figure). This ATP synthase/PIC/ANC supercomplex has been named the "ATP synthasome" and is currently being subjected to structural studies. (*right*) Potential medical application. In other studies, Ko has led a translation project (Ko *et al*., 2004) in which it has been shown that advanced aggressive cancers growing in animals can be completely eradicated by selectively destroying ATP production.

could be forced to rotate in reverse by the electrochemical proton gradient to drive ATP synthesis. In fact, this was the general prediction of the earlier alternating catalytic site model of Gresser *et al*. (1982) noted above. To test this view, a team of investigators at the Tokyo Institute of Technology, Yokohama, Japan inspired by M. Yoshida (Fig. 2(A), bottom right) tethered the F_1 catalytic unit upside down on a glass plate after attaching a fluorescently labeled actin filament to the γ subunit. After ATP was added to initiate ATP hydrolysis by the F_1 catalytic sites, the actin filament attached to the γ subunit was observed via a camera to rotate (Fig. 2(A), top right) indicating that the γ subunit itself must be rotating at the expense of ATP hydrolysis by F_1 (Noji *et al*., 1997). This and many subsequent experiments by this group (reviewed in Yoshida *et al*., 2001) have provided compelling evidence that the mitochondrial ATP synthase is one of nature's smallest machines. In addition, in another project executed at the same location, it has been demonstrated more recently that the V-type ATPases are also molecular machines in which subunit rotation plays an important role (Imamura *et al*., 2003).

The above work spanning over two decades of research by investigators located on three different continents (North America, Europe, and Japan) ushered in a new era of bioenergetics that is likely to be central to biomedical nanotechnology of the future. It also led to Paul Boyer and John Walker sharing half the 1997 Nobel Prize in Chemistry with Jens Skou, the discoverer of the first transport ATPase. Nevertheless, 8 years later the job of obtaining the complete structures of ATP synthase complexes remains far from over, and one should note from the above described work that all investigators, including the author and his colleagues were working only with the headpiece, i.e., the F_1 ATPase part of the ATP synthase molecule. In animal systems the complete ATP synthase contains not just the five subunit types that comprise F_1, but 10 additional subunit types (i.e., a, b, c, d, e, f, g, oscp, F_6, and A6L). In addition, recent findings by Ko *et al*. (2003) indicate that the mammalian enzyme in its native membrane state is also in complex formation with both the phosphate carrier (PIC) and the adenine nucleotide carrier (ANC). This super ATP synthase/PIC/ANC complex consisting of 17 subunit types has been isolated (Ko *et al*., 2003), named the "ATP synthasome" (Fig. 2(B), left panel), and a low resolution structure has been obtained using image reconstruction of single molecules (Chen *et al*., 2004). Future work will be directed at obtaining a three-dimensional structure at atomic resolution of the entire ATP synthasome as this will allow a more complete understanding of the mechanism by which mitochondria make ATP. Using yeast, Stock *et al*., 2000 have obtained a 3.9 Å structure of a F_1-subunit c_{10} complex, a very encouraging start but still lacking two thirds of the subunit types of the complete ATP synthasome.

Aside from all elaborate architecture described above that evolution has molded together into a beautiful machine, the major purpose of the final product, i.e., the ATP synthasome, is to allow biological systems to take ADP, P_i, and Mg^{++} and make MgATP at catalytic sites that reside predominantly on β subunits with some interaction with α subunits. To better understand how such a complex machine may operate to achieve the final product MgATP, it would be helpful to consult one or more recent reviews (Pedersen *et al*., 2000; Stock *et al*., 1999; Weber and Senior, 2003).

How such work described above might be related to "medicine," a word used in the title of this introductory review, is not always obvious. Nevertheless, from the recent work of Ko *et al*. (2004), it is strikingly clear that selectively blocking the ATP production factories of very advanced cancers in animals can result in their complete eradication without bringing about noticeable harm to the animals (Fig. 2(B), right panel). In an apparently unprecedented study, this was the case for 19 out of 19 animals bearing grossly advanced cancers upon treatment with the agent 3-bromopyruvic acid (3-BrPA). This small alkylating agent, a lactic acid analog, preferentially enters aggressive liver cancer cells (hepatocellular carcinoma cells) relative to normal liver cells, hepatocytes. Once inside the cancer cells, it inhibits ATP production by both mitochondria and glycolysis, the former likely at the level of the ATP synthasome and the latter at the level of one or more glycolytic enzymes. The cancers do not return and the animals resume a normal life.

P, ABC, and V-Type ATPases

This section will be kept brief as many of the more than 20 mini-review articles that follow in this special issue of the Journal of Bioenergetics and Biomembranes are devoted to very recent work on these transport ATPases.

Regarding the P-type ATPases, the work reported during the past 5 years on the Ca^{2+} ATPase (SERCA1a) spearheaded by Chikashi Toyoshima at the Institute of Molecular and Cellular Biosciences, The University of Tokyo provides one of the most ideal structural frameworks for understanding the reaction mechanism of a transport ATPase (Toyoshima *et al*., 2000; Toyoshima and Mitusani, 2004). Those investigators who work on

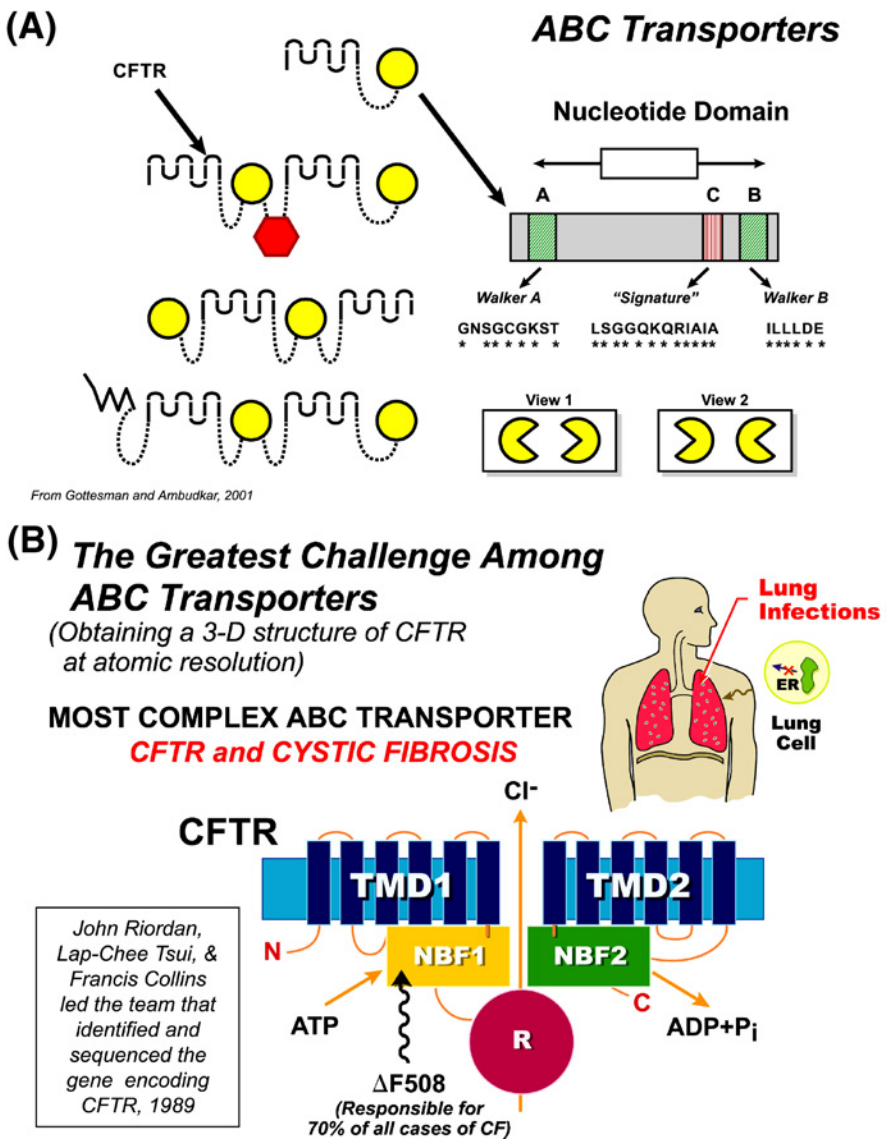

Fig. 3. (A) Simplified diagram illustrating the types of ABC-Type ATPases (ABC transporters) that have been found in biological systems. This figure modified from Gottesman and Ambudkar (2001) shows that ABC transporters discovered to date are usually single polypeptide chains that most frequently contain two homologous but different nucleotide binding domains (*yellow*). These are abbreviated as NBD or NBF. One of these domains is usually located internally within the sequence, while the other depending on the transporter may be localized within the N-terminal region or the C-terminal region. In addition, ABC transporters usually contain two membrane spanning domains. These are abbreviated as MSD or TMD. Much current evidence (see text) suggests that when these transporters are provided with the biological molecule (substrate) that they are supposed to transport, its binding induces the nucleotide binding domains to interact, inducing ATP hydrolysis. The events that follow are not entirely clear despite the availability of several crystal structures, but the hydrolytic events are most likely associated with movements in the MSDs that allow the substrate to move either in or out of a cell, or an organelle, depending on where the ABC transporter is located. There has been much controversy over how the two NBDs interact to stimulate ATPase activity, the extent of this activity, and how the energy of hydrolysis is transmitted to the MSDs. (B) The Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). This transporter has received considerable attention over the past 15 years, i.e., since the transporter was cloned and sequenced. The interest in this protein stems from the fact that numerous mutations, particularly in the two NBDS (NBFs) cause the disease cystic fibrosis that is characterized by chronic lung infections. One of these mutations is a ΔF508 mutation located within NBD1 (NBF1) that is responsible for nearly 70% of all cases of the disease. This mutation causes CFTR to remain in the endoplasmic reticulum, presumably because of a localized problem in folding, rather than trafficking to the plasma membrane where it is thought to function primarily in chloride conduction, although other roles of CFTR have not been ruled out. CFTR is further complicated by the fact that it has a rather large regulatory domain ("red" in A and B in the figure) that can be phosphorylated to different extents that effect the degree of chloride conduction. For all of these reasons noted here it is very important to obtain a 3-D structure of the whole protein, and as indicated in the figure, this author considers this to be "The greatest challenge among ABC Transporters."

this transport ATPase have been blessed with a membrane enzyme consisting of a single polypeptide chain with no additional interacting subunits that at the expense of ATP hydrolysis can translocate 2 Ca^{2+} from the cytoplasm to the lumen of the sarcoplasmic reticulum in muscle. This event is necessary as part of the relaxation process following muscle contraction. Although structures of the Ca^{2+}-ATPase have been obtained for five different states by X-ray crystallography some still remain to be published. However, the implications from most of this work as it relates to the mechanism of Ca^{2+} transport is discussed in a detailed review written recently by Toyoshima and Inesi (2004). Significantly, the most intriguing features about this transport ATPase relate to the dynamics of its various domains during Ca^{2+} transport from the cytoplasm to the lumen of the sarcoplasmic reticulum, and how Ca^{2+} becomes occluded and eventually is released. Those who have watched the past acts of Houdini, the famous magician who subjected himself to entrapments of all sorts and then with rapid and large dynamic movements released himself, will greatly enjoy the mechanism of this P-Type ATPase. Moreover, it seems likely that most other P-Type ATPases will undergo similar types of "Houdini-like" mechanisms.

Regarding ABC-Type ATPases (ABC transporters), the largest class of transport ATPases (Higgins, 1992; Higgins *et al*., 1986; Hyde *et al*., 1990), many share in common with the Ca^{2+}-P-Type ATPase the structural feature that their relevant domains frequently occur on a single polypeptide chain, although sometimes on separate shorter polypeptides that dimerize (Fig. 3(A)). For those that exist as single polypeptide chains, they fold into two membrane spanning domains (MSDs) and two nucleotide binding domains (NBDs or NBFs) located in the cytoplasm. Therefore, similar to P-Type ATPases with single polypeptide chains, these ABC transporters must do the job of transporting through a membrane a given substance or ion at the expense of ATP hydrolysis. However, the 4 domain arrangement (2 MSDs and 2 NBDs) suggests a different mechanism. Now that several of these ABC transporters have been crystallized at or near atomic resolution (Chang and Roth, 2001; Chen *et al*., 2003; Locher, 2004), some insights into this mechanism are beginning to evolve. One view is that when the conformation of the ABC transporter is such that the two NBDS remain separated (don't touch) a transmembrane translocation pathway is open to the cytoplasm, perhaps near the center of the two MBDs. The substrate to be transported is then bound bringing the two NBDs together and stimulating both ATP hydrolysis and conformational changes that promote transport of the substrate.

Certainly, to understand how ABC transporters work there will need to be more structures particularly from mammalian systems where disease causing mutations frequently occur. Perhaps the biggest experimental challenge in the field of ABC transporter research is to obtain several atomic resolution structures of the complete cystic fibrosis transmembrane conductance regulator (CFTR), the ABC transporter/channel that translocates chloride (Cl^-) through the plasma membrane of mammalian cells and in which numerous mutations have been shown to cause the disease cystic fibrosis (Fig. 3(B)). It will be noted that CFTR in addition to containing two MSDs and two NBDs, also contains a regulatory domain designated simply as "R" that when phosphorylated promotes conduction of Cl^-. Therefore, in any future structural study that focuses on CFTR, it is important to try to obtain crystals and a subsequent 3-D structure at atomic resolution of the complete molecule that includes the R domain. Recently Awayn *et al*. (2005) have obtained two dimensional crystals of CFTR, and from these, a low resolution (20 Å) map of the whole protein. This is a very encouraging indication that 3-D crystals may be obtained in the future and could lead to an atomic resolution structure of the complete CFTR protein.

Finally, as it relates to the mechanism of the V-Type ATPases, the similarities of the coated vesicle enzyme to F-Type ATPases (ATP synthases) from mammalian cells in terms of overall structure (Wilkens *et al*., 2005) and sub-structure (Forgac, 2000) strongly implicates the V and F-Type ATPases exhibiting some mechanistic commonalities as it relates to ATP hydrolysis and ATP-dependent proton translocation. This suggestion gains additional support from the recent finding, that similar to F-Type ATPases, the V-Type ATPases also catalyze ATP-dependent subunit rotation (Imamura *et al*., 2003; Hirata *et al*., 2003; Yokoyama *et al*., 2003). Future crystal structures at atomic resolution of the complete F-Type and V-Type ATPases should define very clearly the commonalities and differences between these two remarkable motor enzymes.

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The author is most grateful for grant awards that he has received from the NIH to support both his basic research on transport ATPases and his research relating the former to disease and medicine. The author also thanks all of those who have contributed photographs. Figure 1 (bottom half) of this review and the cover Figure represent and updated modified version of Figure 1 from Pedersen, P. L., *J. Bioenerg. Biomemb.* **34**, 327–333, 2002. Figure 2

(upper right, i.e., rotating γ subunit of F_1 ATPase) is a color modified version (provided by M. Yoshida) for the cover Figure of the J. Bioenerg. and Biomember. 29–3. The 3-dimensional structures presented in the upper center part of Figure 1 are referenced in the figure and color modified from available structural information (pdb codes 1 MAP and 1 E79). The bottom part of Figure 2 (lower left) showing the mitochondrial ATP synthasome complexed with antibodies to the phosphate carrier (PIC) and the adenine nucleotide carrier (ANC) was reproduced from the lower parts of Figs. 1D and 1E in Chen, C., Ko, Y. H., Delannoy, M., Ludke, S. J., Chiu, W., and Pedersen, P. L., *J. Biol. Chem.* **279**, 31671–31678. (Permission was obtained for its reproduction from the American Society for Biochemistry and Molecular Biology). Figure 2, lower right, depicting a tumor treated animal was obtained from Figure 2, of Ko, Y. H., Smith, B. L., Wang, Y., Pomper, M. G., Rini, D. A., Torbenson, M. S., Hullihen, J., and Pedersen, P. L., *Biochem. Biophys. Res. Commun.* **324**, 269–275, 2004. (Permission for its reproduction was obtained from Elsevier.) Figure 3A of was adapted in part from Figures 1 and 2 of Gottesman, M. M., and Ambudkar, S. (2001) *J. Bioenergetics and Biomembranes* **33**, 453–458. Finally, the author pays special thanks to all the discoverers of transport ATPases for without these discoveries all those mentioned here would likely be in a less exciting profession.

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