... and biologists' work on protein energy converters

[LONDON] The 1997 Nobel prize for chemistry has been awarded to three biologists for their work on proteins that interconvert chemical energy, in the form of the 'high-energy' compound adenosine triphosphate (ATP), and electrical energy — the asymmetric distribution of ions across biological membranes.

Jens Skou of Aarhus University, Denmark, receives half the prize for his discovery of the Na^+/K^+ pump. Paul Boyer of the University of California, Los Angeles, and John Walker of the UK Medical Research Council's Laboratory of Molecular Biology in Cambridge share the other half for their elucidation of the mechanism of ATP synthase. Between them, these three straddle more than 40 years of research in bioenergetics.

Although few doubt the researchers fully deserve such recognition, many were surprised by their presence on the same citation and by the timing of the award to Boyer and Walker. Many questions about the mechanism of ATP synthase — in particular its complete structure — remain unanswered.

The distribution of the prize has also raised some eyebrows. Work on the sodium pump was "long overdue for recognition", says J. Clive Ellory of the University of Oxford's physiology department. But it had been expected that Skou would eventually share the prize with other prominent researchers in the field, such as Ian Glynn of the University of Cambridge and Robin Post of Vanderbilt University, Tennessee, who helped characterize the workings of the Na^+/K^+ pump.

Enzymes and energy

Skou's pioneering work in the late 1950s showed that an enzyme could take the energy supplied by ATP and use it to transport material across a cell's membrane. It had just been established that different concentrations of ions were maintained inside and outside cells, and Skou set about identifying the cause of this disparity by analysing the nerves of crabs, extracting cell membranes in which he identified an ATP-degrading enzyme stimulated by sodium ions.

Further investigation showed that this protein comprised two subunits and, at the cost of one molecule of ATP, moved three sodium ions from the inside of the cell to the outside and two potassium ions from the outside to the inside.

Boyer and Walker attacked a reciprocal problem. Rather than study how ATP creates ion gradients across cell membranes, they looked at how the energy in these gradients can be used to synthesize ATP from its low-

The three winners of the Nobel prize for chemistry: Walker (above) Skou (top right) and Boyer (left), together cover a span of more than 40 years of research in bioenergetics.

energy form, adenosine diphosphate (ADP).

Peter Mitchell, working at the Glynn Laboratory in Bodmin, Cornwall, had shown in the 1960s that the ultimate result of metabolism — the breakdown of an organism's food source — was to produce a difference in hydrogen ion concentration across the cell's mitochondrial membrane, work for which he received the Nobel prize in 1978.

Boyer worked through the 1970s and 1980s investigating ATP synthase, the enzyme that uses this gradient to synthesize ATP. It was soon discovered that this enzyme consisted of a large number of proteins, but that it could be separated into two parts: F_0 , which was tightly bound in mitochondrial membrane, and F_1 , which contained the ATP binding and synthesizing activity.

Concentrating on F_1 , Boyer showed that it was made of three copies, each of two similar subunits, α and β , of which β bound the ATP. But the subunits were never in the same state — one bound ATP and one ADP, while the third had an empty binding site.

Boyer proposed that the subunits changed between these conformations in a concerted fashion, constantly cycling from empty to ADP-bound to ATP-bound in what he called the "binding change mechanism".

There was one other component of F_1 , the γ -subunit, of which there was only one copy. Boyer proposed that this γ -subunit rotated within the other subunits like a crankshaft in a three-cylinder motor. "Why Paul's achievement is so praiseworthy," according to Richard Cross of the State University of New York Health Science Center, is that these "parts of the mechanism flouted dogma".

Indeed, it has taken almost all of the 16 years since the mechanism was fully laid out to establish its veracity. The greater part of this proof has come from Walker, who started out in the 1980s to provide a clear picture of the enzyme and its action.

He began by determining the sequences of the various subunits using low-resolution electron microscopy, which showed a 'lollipop'-shaped protein sticking out from the mitochondrial membrane. By 1994, he had elucidated the atomic detail of F_1 using X-ray crystallography (see *Nature* 370, 621–628; 1994). This structure more than bore out the predictions of Boyer, showing an elongated γ -subunit pushing against one of the α/β pairs, forcing the three to adopt their differing conformations.

Still unknown are the structure and action of F_0 , which somehow uses a flow of hydrogen ions across the mitochondrial membrane to drive the rotation of F_1 . Work is well under way to determine this structure, and it would be unwise to bet against Walker solving this as well.

Without such information, has the Nobel committee been premature in its award? David Hackney of Carnegie Mellon University, Pittsburgh, is certain that it has not. "It is recognition of something that Paul [Boyer] has worked his entire life on," he says. "All his propositions, which were controversial at the time, have turned out to be true."

Demonstration of rotation

The most controversial of these propositions was that F_1 rotates and it may be that a graphic demonstration of this earlier in the year triggered the presentation of the prize.

Richard Cross and Wolfgang Junge of Osnabrück University had separately provided indirect evidence for the rotation, but it was Masasuke Yoshida of the Tokyo Institute of Technology who, by attaching a filament to the γ -subunit, was able to film the rotation in action (see *Nature* 386, 299–302; 1997). Cross is convinced this was a great influence on the Nobel committee. "When he [Yoshida] showed that film at meetings, people's jaws dropped," he said.

Hackney recalled it as the "only time that a platform session [was] stopped due to applause from the floor". He has no doubt about its impact. "The biochemical data were sufficient to convince people in the field [about Boyer's mechanism] but the visualization of the spin was the last nail to finalize it."

What are the prospects of the same level of structural information emerging to illuminate Skou's Na^+/K^+ pump? Gene Scarborough of the University of North Carolina is working on the problem and hopes a structure will emerge within five years. He has no doubts about the rightness of the award to Skou. "When I teach this stuff [bioenergetics] I always begin with Skou, because that's where it begins."

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