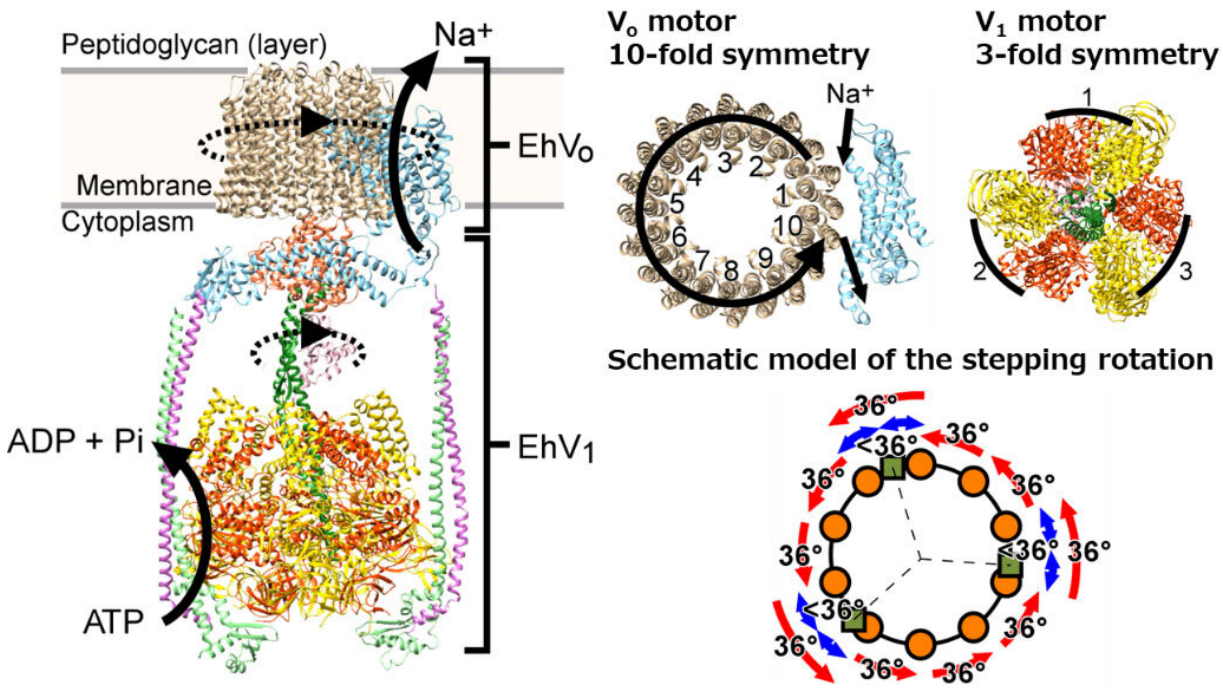
# Molecular-motor specialists deepen our understanding of the rotary ion pump of the cell

October 13 2022




Structure of EhVoV1 and schematic model of stepping rotation. Credit: Ryota Iino, Institute for Molecular Science, National Institutes of Natural Sciences

A team of specialists in nano-sized rotational motors have directly visualized the process of pumping sodium ions, enabling them to explain why there had up until now appeared to be a structural symmetry

mismatch between two motors that make up part of the key protein driving the process. Their findings should help develop a better understanding of the mechanisms involved with cellular energy-conversion motors more generally.

A paper describing their investigation appeared in the *Proceedings of the National Academy of Sciences* on October 10, 2022.

Many will remember from secondary-school biology how in complex cells, a protein called ATP synthase—embedded in the membrane of the mitochondria, often described as the powerhouse of the cell—operates sort of like the way that a hydroelectric plant works, but to produce usable energy for an organism. A hydroelectric plant exploits the rush of water coming from the reservoir behind a dam to turn a turbine that drives a dynamo that produces electricity.

Similarly, the "rotors" of an ATP synthase protein use a rush of protons from a region on one side of the membrane with a high proton concentration to a region on the other side of low concentration (a "proton gradient") to rotate a "stalk" within the ATP synthase that manufactures molecules of adenosine triphosphate (ATP)—the energy currency of the cell that is used to power its activities.

There is also an "opposite" protein to the ATP synthase called vacuolar ATPase (V-ATPase) that operates as a proton "pump" that uses the energy from ATP to produce a proton gradient. (In a similar way to how some hydroelectric plants that operate like large batteries can work in reverse, using electricity to pump water back up into a reservoir)

V-ATPases consist of two rotary motors, $V_o$ and $V_1$, and earlier studies had been conducted on model types of V-ATPases to understand how these two motor proteins are able to couple their rotational motions and functions.

However, few studies have been performed on other types of V-ATPases that may have slightly different functions and structures of their component parts. A comprehensive understanding of the mechanism converting one type of energy into another remained elusive.

Furthermore, V-ATPases can pump not just protons, but also sodium ions ($Na^+$) as well. In the bacterium Enterococcus hirae (E. hirae—which sometimes causes sepsis in humans), its V-ATPase works as an ATP-driven $Na^+$ pump to maintain desired sodium ion concentrations inside the cell.

What was known about the heretofore under-investigated E. hirae V-ATPase is that a unit of the ATP energy currency is "spent" (hydrolyzed), driving rotation of its $V_1$ motor ($EhV_1$; the other motor is called $EhV_o$). But it does this in stepwise fashion. $EhV_1$ rotates only 120° per "expenditure" of ATP, meaning that it takes 3 ATP molecules to rotate a full 360°.

In addition, previous research by the scientists had found that each 120° step of the $EhV_1$ rotor is further divided into sub-steps of 40° and 80° (adding up to 120°). But the scientists had been unable to clearly resolve quite how steps and related pauses of $EhV_o$ in rotation occurred.

The key mystery was a mismatch between the two motors. ATP hydrolysis generates rotational torque in the $EhV_1$ motor, which is then translated to the $EhV_o$ motor. The latter motor also has a subunit composed of a ring of ten subunits ($c_{10}$-ring), which rotates in the membrane. The sodium ions are transported across the membrane by the rotation of this $c_{10}$-ring.

"But there's a structural symmetry mismatch here: the ratio between the number of sodium ions transported and the number of ATP spent per rotation," said Ryota Iino, a biophysicist and professor from the Institute

for Molecular Science of Japan's National Institutes of Natural Sciences.

"There are ten sodium ions transported per full turn, and each full turn costs three ATP, or one ATP per 120° step of the $EhV_1$ motor," Professor Iino continued. "Ten divided by three obviously equals 3.3, or one and a third sodium ions. But you can't have a third of a sodium ion. So what's going on?"

The scientists knew that the rotational coupling of the motors in ATPases in E. coli bacteria and yeast had some "give" or were what they term "elastic." In these organisms, the symmetry mismatch is relieved either by large deformations in the peripheral stalk of the protein or in a central rotor. On the other hand, it could be the case that due to the multiple peripheral stalks of the E. hirae V-ATPase, the coupling between motors could be rigid rather than elastic, and so the mismatch would likely then be resolved through additional pauses during rotation.

The only way to find out which of the two likely options was happening—or combination of the both—was via direct visualization of such steps and possible pauses involved in the E. hirae V-ATPase motors' movement.

Using a 40 nanometer nanoparticle of gold as a probe that could be tracked with a high-speed camera, the researchers found that the binding of sodium ions and binding of ATP to different parts of the protein occur at different angles, and the coupling between the two motors does indeed involve a rigid component. The rigid component in turn requires 13 pauses—resolving the mismatch (and need for a "third" of a sodium ion), as well as occasional backward steps in the process.

The results also confirm that V-ATPases, with their multiple peripheral stalks, are more rigidly coupled than the ATPases that only have one peripheral stalk and work as ATP synthases.

The team expects their imaging of the V-ATPase motors in action will pave the way for a comprehensive understanding of the mechanisms involved with ATPases more generally.

**More information:** Akihiro Otomo et al, Direct observation of stepping rotation of V-ATPase reveals rigid component in coupling between V o and V 1 motors, *Proceedings of the National Academy of Sciences* (2022). DOI: 10.1073/pnas.2210204119

Provided by National Institute for Physiological Sciences