A new model for an old friend

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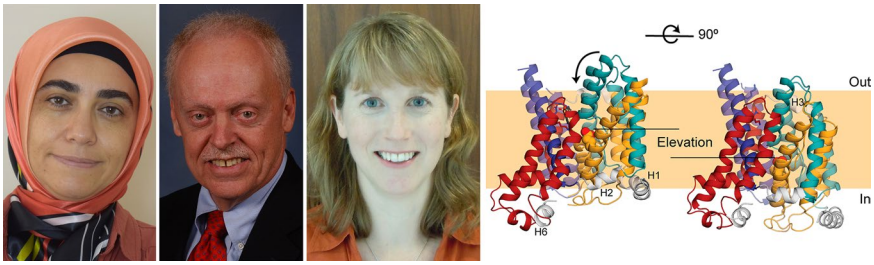
JGP study suggests the anion exchanger AE1 operates via an elevator-like mechanism.



First author Emel Ficici (left) and collaborators José D. Faraldo-Gómez (not shown), Michael L. Jennings (middle), and Lucy R. Forrest (right) applied repeat-swap modeling to the AE1 anion exchanger to obtain a hypothetical inward-facing conformation of the protein (see ribbon diagram) and infer its mechanism of ion exchange.

Ion transport across cell membranes is essential for many metabolic and biochemical processes. For example, in red blood cells, the protein AE1 (also known as band 3) exchanges extracellular chloride for intracellular bicarbonate to support CO_2 transport in blood. In this issue of JGP, Ficici et al. provide a potential explanation for how AE1 might work (1).

AE1 is a dimer composed of two identical subunits. Each subunit contains an N-terminal domain that resides in the cytoplasm and a C-terminal domain, spanning 14 transmembrane helices, which accomplishes both dimerization and ion transport. Like other exchangers, AE1 is thought to operate using an "alternating access" mechanism, whereby transported ions can bind from either the extracellular side of the protein or the intracellular side, but not both at once.

"Two major conformational mechanisms for alternating access have been identified: one mechanism in which one or more domains of the protein rock around the binding site to open a pathway to it from either side, and another where an entire domain carries the substrate from one side of the membrane to the other," explains Lucy Forrest, a Senior Investigator at the US National Institute of Neurological Disorders and Stroke. Evocatively, the first is called the "rocking" transport mechanism; the second is termed "elevator-like." Which does AE1 use?

Previous studies inferred AE1's transport mechanism based on its homology to other proteins, but these attempts produced conflicting conclusions (2, 3). Fortunately, a crystal structure for AE1 is available (4), which shows the protein in a conformation with its ion-binding site open to the extracellular side of the membrane (i.e., in an outward-facing conformation). It isn't evident from the structure what transport mechanism AE1 uses, but the structure's publication sparked an idea for Michael Jennings, a Professor at the University of Arkansas for Medical Sciences who has studied AE1 for years.

"I became interested in the possibility of using repeat-swap modeling to try to determine what the inward-facing state of this protein looks like, so I contacted Lucy and asked if she was interested in doing the modeling," he says.

Repeat-swap modeling has been used to successfully model other transporters that use rocking (5) and elevator-like transport mechanisms (6). AE1 was a good candidate for repeat-swap modeling because the C-terminal domain structure meets the preconditions for using this approach: it contains repeating structural elements that are topologically inverted with respect to each other and exhibits structural asymmetry between those inverted repeats. These characteristics allow researchers to model the first repeat based on the structure of the second, and vice versa.

"If the asymmetry between those repeats is responsible for the fact that the structure is outward-facing in the first place, then when you exchange their conformations, the structure becomes inward-facing. This process effectively reveals a new pathway to the other side of the membrane," notes Forrest.

Emel Ficici, a postdoc in collaborator José Faraldo-Gómez's laboratory at the National Heart, Lung, and Blood Institute, performed the modeling to obtain a snapshot of what AE1 might look like when its ion-binding site is exposed to the cytoplasmic side of the membrane. Next, to explore AE1's mechanism of action, Ficici et al. used linear interpolation to infer how the protein might transition between its outward- and inward-facing conformations. Interestingly, although AE1 doesn't structurally resemble other elevator transporters, this work indicated that AE1 likely uses an elevator-like mechanism for transport.

Because AE1 is well studied, there exists a wealth of data describing the functional effects of mutations, cleavage and cross-linking residues within AE1. "As we discuss in our paper, many of these data fit very well with our prediction," observes Forrest.

Forrest and Jennings stress that validation of their hypothesis awaits additional structure/function studies, but hope that their repeat-swap model will help researchers better understand AE1 and related proteins.

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