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Protons and how they are transported by proton pumps

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Abstract The very high mobility of protons in aqueous solutions demands special features of membrane proton transporters to sustain efficient yet regulated proton transport across biological membranes. By the use of the chemical energy of ATP, plasma-membrane-embedded ATPases extrude protons from cells of plants and fungi to generate electrochemical proton gradients. The recently published crystal structure of a plasma membrane H^+-ATPase contributes to our knowledge about the mechanism of these essential enzymes. Taking the biochemical and structural data together, we are now able to describe the basic molecular components that allow the plasma membrane proton H^+-ATPase to carry out proton transport against large membrane potentials. When divergent proton pumps such as the plasma membrane H^+-ATPase, bacteriorhodopsin, and F_OF_1 ATP synthase are compared, unifying mechanistic premises for biological proton pumps emerge. Most notably, the minimal pumping apparatus of all pumps consists of a central proton acceptor/donor, a positively charged residue to control pK_a changes of the proton acceptor/donor, and bound water molecules to facilitate rapid proton transport along proton wires.

Keywords Proton transport · P-type pumps · Membrane potential · Membrane protein structure · Membrane transport · Proton current · Topical review · Protons · ATP

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Proton transport across biological membranes

Compared to other ions, protons have an extremely high mobility in aqueous solution. Protons in aqueous solution do not exist as naked protons, but are instead found as hydrated proton molecules, either as a H_5O^{2+} dihydronium ion complex or as a $H_9O_4^+$ complex (with a hydronium ion, H_3O^+, hydrogen bonded to three water molecules) [3, 10, 31]. The high proton conductance of water can be ascribed to the movement of protons along hydrogen-bonded networks of water molecules. A linear hydrogen-bonded chain of water molecules that can carry out proton transfer is termed a proton wire. The ability of protons to rapidly propagate along a proton wire involves jumping of protons from one water molecule to the next along the wire [4, 8]. In proteins, elements of a proton wire can also be protonable amino acid residues [8]. In this way, the diffusion of protons in solution and their interaction with other molecules such as proteins are mechanistically different from that of cations [14]. In a fixed proton wire, excess protons that enter the wire will cause proton propagation along the wire, and a proton entering the wire is not the same as the proton leaving it.

In gramicidin, a membrane-embedded proton channel, the proton wire is constituted of water molecules that line the walls of the channel [9, 26]. A proton can travel the

length of the gramicidin channel in a much shorter time span than the catalytic mechanism of any known proton pump allows [3]. Therefore, proton pumps that transport protons against an electrochemical gradient must be in control of mechanistic devices that prohibit channel formation and proton leakage through the protein. Such short circuit of the system can be prevented either through conformational changes that break the continuity of the proton wire during catalysis or by the introduction of electrostatic barriers along the proton pathway [3].

In bacteriorhodopsin, the best characterized proton pump protein, an ordered chain of water molecules, protonatable aspartic and glutamic acid carboxyl groups, and a prosthetic group, comprise the directional proton wire and the proton pumping apparatus [13, 15–18, 20]. However, there is no continuous proton wire throughout the pump molecule as a central proton acceptor/donor causes a disruption of the proton wire into two halves. A carboxyl group at this intramembranous location, namely Asp85, is protonated in response to receipt of light by the prosthetic group. Control of the pK_a of Asp85 is an essential part of proton transfer. Thus proton release from Asp85 is driven by shuttling the positive charge of the nearby residue Arg82 between the up and down positions, which again affects the pK_a of Asp85 [17].

F_OF_1 ATP synthase is a proton pump with a very different architecture from that of bacteriorhodopsin [7, 11, 12]. However, the general scheme for proton transport in these two pumps seems to be the same: A proton coordination centre buried in the membranous part of the pump serves as an acceptor for protons delivered via a proton wire. The same centre donates bound protons to an exit pathway, a proton wire leading towards the other side of the membrane. In F_oF_1 ATP synthase, ATP synthesis is accomplished when protons at the side of the membrane with high local proton concentration bind to a low pK_a site (Asp61 in the c subunit of the F_oF_1 ATPase), are translocated, and then released to the side with low proton concentration. The *c*-subunits are organized in a ring that turns to allow interaction of Asp61 with the Arg210 residue of subunit a. Contact between these two residues lowers the pK_a of *c*Asp61 to enable ionization and H^+ release to the exit channel [12].

Plasma membrane proton pumps

A trans-plasma membrane electrochemical gradient of either protons or sodium is required for all organisms to take up nutrients by secondary active transport systems. In most life forms, plasma-membrane-localized proton pumps, or H^+-ATPases, energize the cell membrane, with the exception of animals that depend on the Na^+/K^+-ATPase for this purpose [22]. Plasma membrane proton pump genes were first cloned from fungi [27] and plants [23], and later on identified in archaebacteria and protists [2]. Plasma membrane proton pumps belong to a large superfamily of pumps termed P-type ATPases [24].

A hallmark of P-type ATPases is the formation of a phosphorylated intermediate during catalysis [24]. P-type ATPases can have additional subunits, but in all P-type pumps ATP hydrolysis and cation transport are carried out by a single catalytic polypeptide. Well-characterized members of this family comprise the plasma membrane H^+-ATPase, the animal Na^+/K^+-ATPase, the animal sarcoplasmic reticulum Ca^{2+}-ATPase, the animal plasma membrane Ca^{2+}-ATPase, and the animal H^+/K^+-ATPase. The catalytic cycle of P-type ATPases is described principally by two main conformational states, E_1 and E_2, which alternate during transport. In the E_1 conformation, the transmembrane binding site(s) have high affinity for the exported ions, whereas in E_2 the same site(s) have low affinity for the exported ions and high affinity for the counter-ions being imported.

In plasma membrane H^+-ATPases, a single proton is believed to be transported per hydrolyzed ATP [22]. There is no evidence that counter-ion transport takes place, in contrast to what is known for the other members of the P-type ATPase studied. Plasma membrane proton pumps can generate very high membrane potentials (up to the order of −300 mV in fungi). This is higher than has been reported for any other transport system and much higher than the potential of approximately −70 mV that is generated by the Na^+/K^+ATPase, the animal homologue to the proton pump. It is of interest to ask why the plasma membrane proton pump appears more efficient when compared to the sodium pump, and what the structural basis is for this difference.

Mechanism of transport

Structural information of plasma membrane P-type H^+-ATPases has been obtained from 2D crystals of *Neurospora crassa* H^+-ATPase [1] and from 3D crystals of plant H^+-ATPase [25].

The recently published E_1 crystal structure of the plant H^+-ATPase offers novel mechanistic insight into the proton transport pathway and the mechanism of proton transport [25] (Figs. 1 and 2). The general folding pattern of the pump is remarkably similar to that of the sarcoplasmic reticulum Ca^{2+}-ATPase [21, 28–29] and the animal Na^+/K^+-ATPase [19]. The catalytic unit of the ATPase molecule includes four domains: N (nucleotide binding), A (actuator), P (phosphorylation), and M (membrane). The cytoplasmically positioned N, P, and A domains are in charge of ATP hydrolysis. Conformational changes in these domains during catalysis lead to simultaneous movements in the

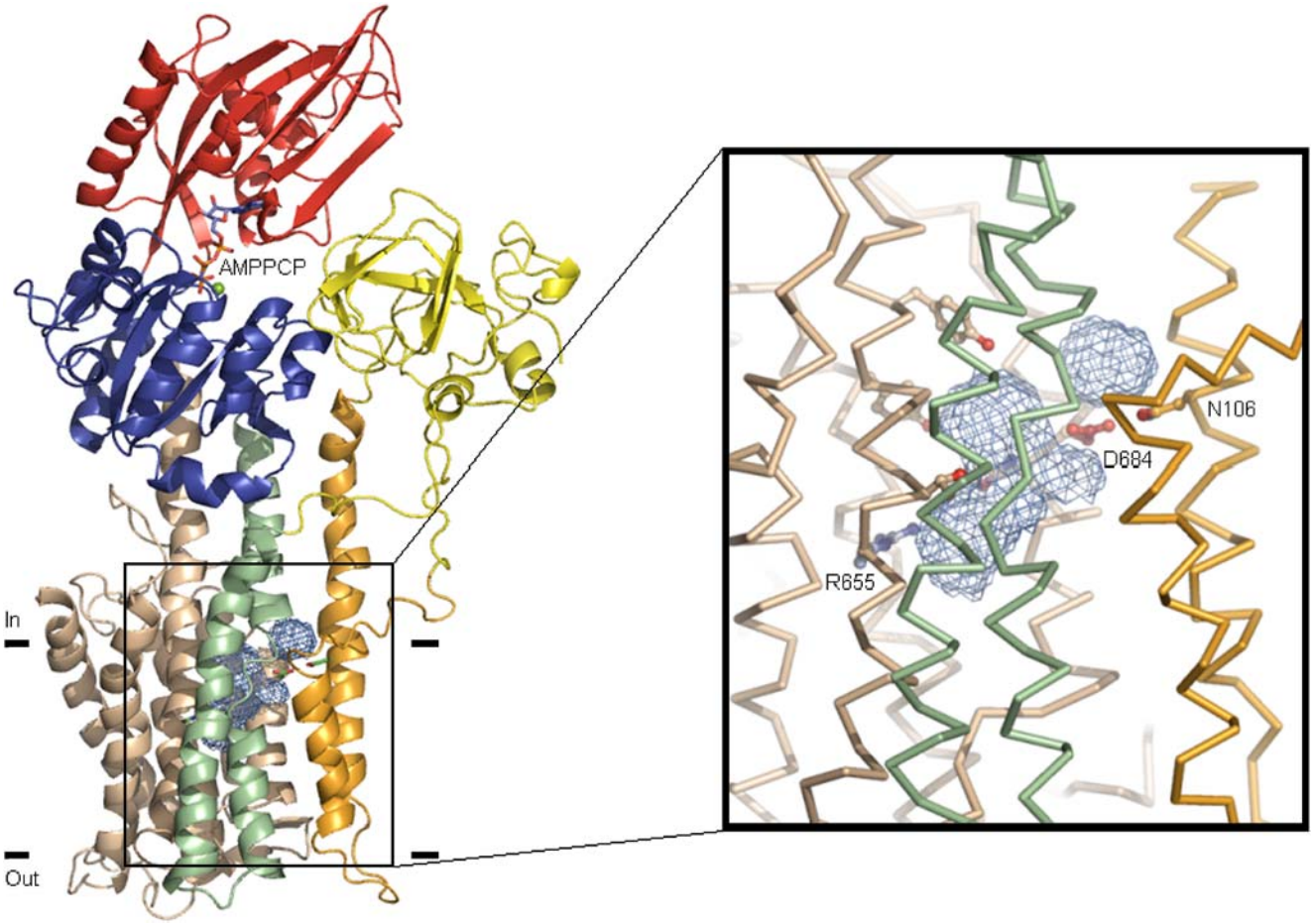

Fig. 1 Structure of the plant plasma membrane H^+-ATPase [25]. The structure represents an active form of the AHA2 proton pump, without its auto-inhibitory C-terminus, in complex with Mg-AMPPCP, a non-hydrolyzable ATP homologue. Ten transmembrane helices (*orange*, *green*, and *brown*), nucleotide-binding domain (*red*), the phosphorylation domain (*blue*), and the actuator domain (*yellow*). Mg-AMPPCP is found at the interface between the N and P domains. *Black lines* depict the expected location of the plasma membrane. The *inset* is an enlarged view of the transmembrane part showing the central cavity of the pump, which is likely to be filled with water. Key residues mentioned in the text are shown as *sticks*. *In*, cytosolic side. *Out*, extracellular side

membrane-embedded part that directs the proton transport. The C-terminal extension of the plasma membrane H^+-ATPase that serves as a flexible regulatory domain cannot be observed in the structure.

The transport site

Although the overall structure of the plasma membrane proton pump is very similar to other structurally characterized P-type pumps, the active players in the transport machinery are unlike [25]. Thus, it seems that similar structural arrangements in P-type pumps have evolved to perform very different tasks.

The transporting unit of the H^+-ATPases is defined by a single, centrally located proton acceptor/donor (Asp684), an asparagine residue (Asn106), a positively charged arginine amino acid residue (Arg655), and a large central cavity likely to be filled with water (Fig. 1). The cavity is lined by polar and charged amino acid residues. The fact that all these residues are highly conserved underscores the importance of the cavity and its internal surface.

Mutational studies had previously pointed to an essential role for Asp684 in proton pumping by the plasma membrane proton ATPase. Removal of its negative charge results in uncoupling of the pump molecule and the complete incapacity of the pump molecule to perform proton transport [5, 6]. In the E_1 crystal structure of the H^+-ATPase, Asp684 is in close contact with Asn106 in accordance with hydrogen bond formation between the side chains of these two residues. In harmony with a proton-bound E_1 structure, this structural arrangement is expected to favor the protonated form of Asp684.

Arg655 is placed at the opposite side of the cavity and is believed to direct pK_a changes of Asp684 during catalysis as an aid in proton release from Asp684 at a later stage in the catalytic cycle. Arg655 is not indispensable for either ATP hydrolysis or proton transport, but the pump is highly sensitive to mutagenesis at this position [6].

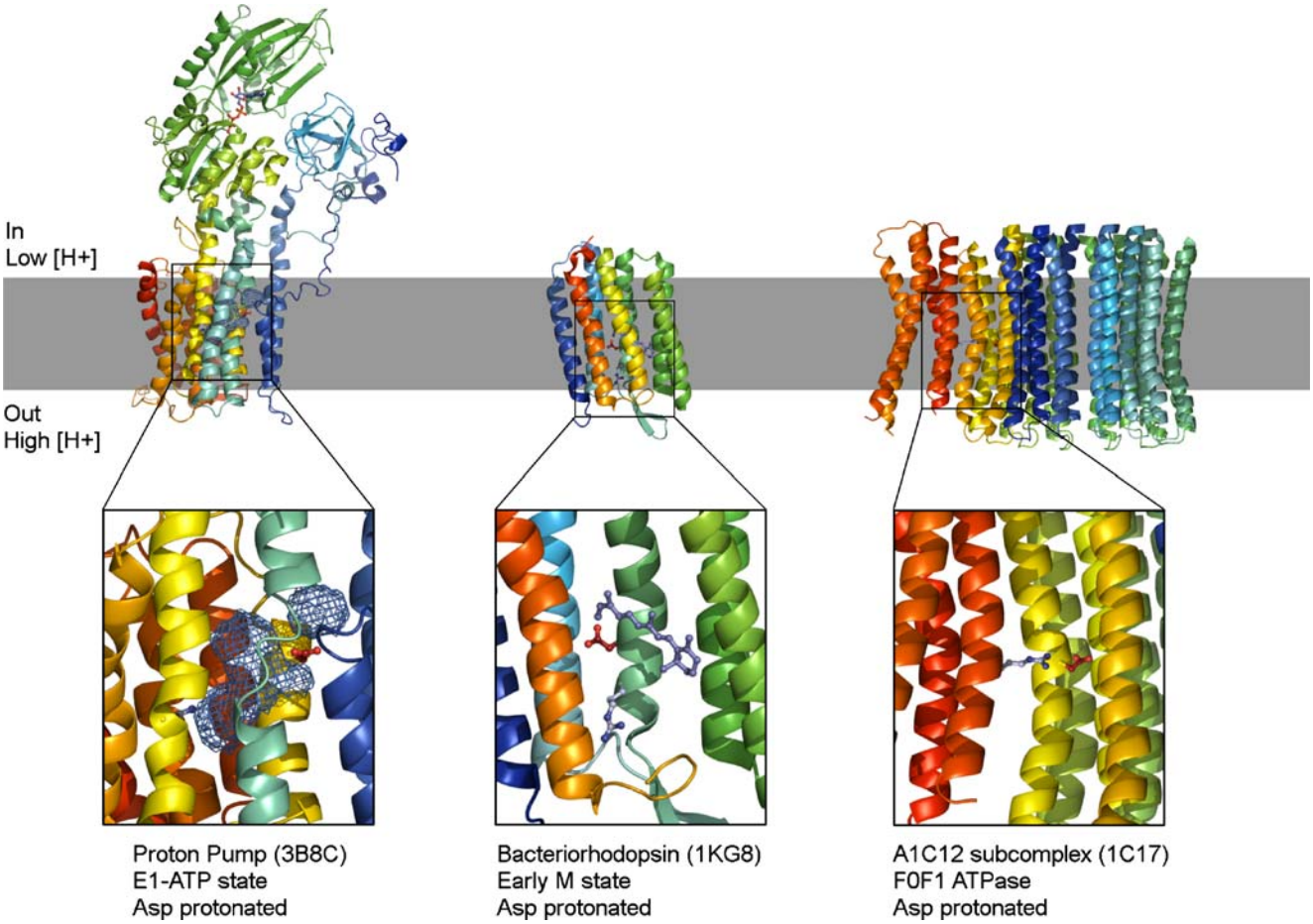

Fig. 2 Structural comparison of pump architecture in different proton pumps. In all pump systems, an essential Asp residue (*shown in red*) serves as a central proton acceptor/donor and a nearby Arg residue (*shown in blue*) is involved in controlling pK_a of the Asp. *Left*, the plasma membrane proton pump. *Center*, bacteriorhodopsin. *Right*, the C ring of F_OF_1 ATPase

Proton loading

A proton inlet channel must be present to direct transported protons to Asp684. In the determined E_1-AMPPCP structure, no proton inlet channel is discernible. However, a single cavity of a size at approximately 80 Å^3 in size is located at a path along the proposed proton inlet channel (Fig. 1). A semi-closed inlet channel is in concurrence with the crystallized quasi-occluded form of the H^+-ATPase. Going from the intracellular compartment, protons would have access to Asp684 when the channel is open in its proton binding conformation. Conserved residues (Glu113, Glu114, Asn106) are positioned in this area of the pump molecule. Here they could participate in creating a directional proton inlet wire leading directly to Asp684 by specific positioning and orientation of water molecules in a hydrogen-bonded network. Asp684 and Asn106 are placed such that they generate a barrier between the proton inlet channel and the central cavity in the structure. Protonation of Asp684 is believed to facilitate hydrogen bond formation between these two, indicative of a gatekeeper role for the protonated Asp684-Asn106 pair. As the hydrogen bond is formed, it is expected to stabilize Asn106 and membrane segment M2. M2 is connected directly to the cytoplasmic A domain. Thus, following proton binding, a signal might be transmitted via M2 to the cytoplasmic domains that invoke phosphorylation. In this way, tight coupling between substrate loading in the transmembrane domain and energy expenditure is obtained. This ensures that a pumping event only takes place when substrate is loaded in the pump. Conversely, formation of the phosphorylation site stabilizes the protonated conformation. This is a general feature of P-type ATPases, well explained in the case of the Ca^{2+}-ATPase and Ca^{2+} occlusion [28, 30].

Proton unloading

Phosphorylation leads to $E_1P \rightarrow E_2P$ conformational changes and proton unloading as the Asn106–Asp684 pair is forced apart. When the conformational change is modeled on the basis of that observed in Ca^{2+}-ATPase [21], the intramembranous cavity opens up to the extracellular side and

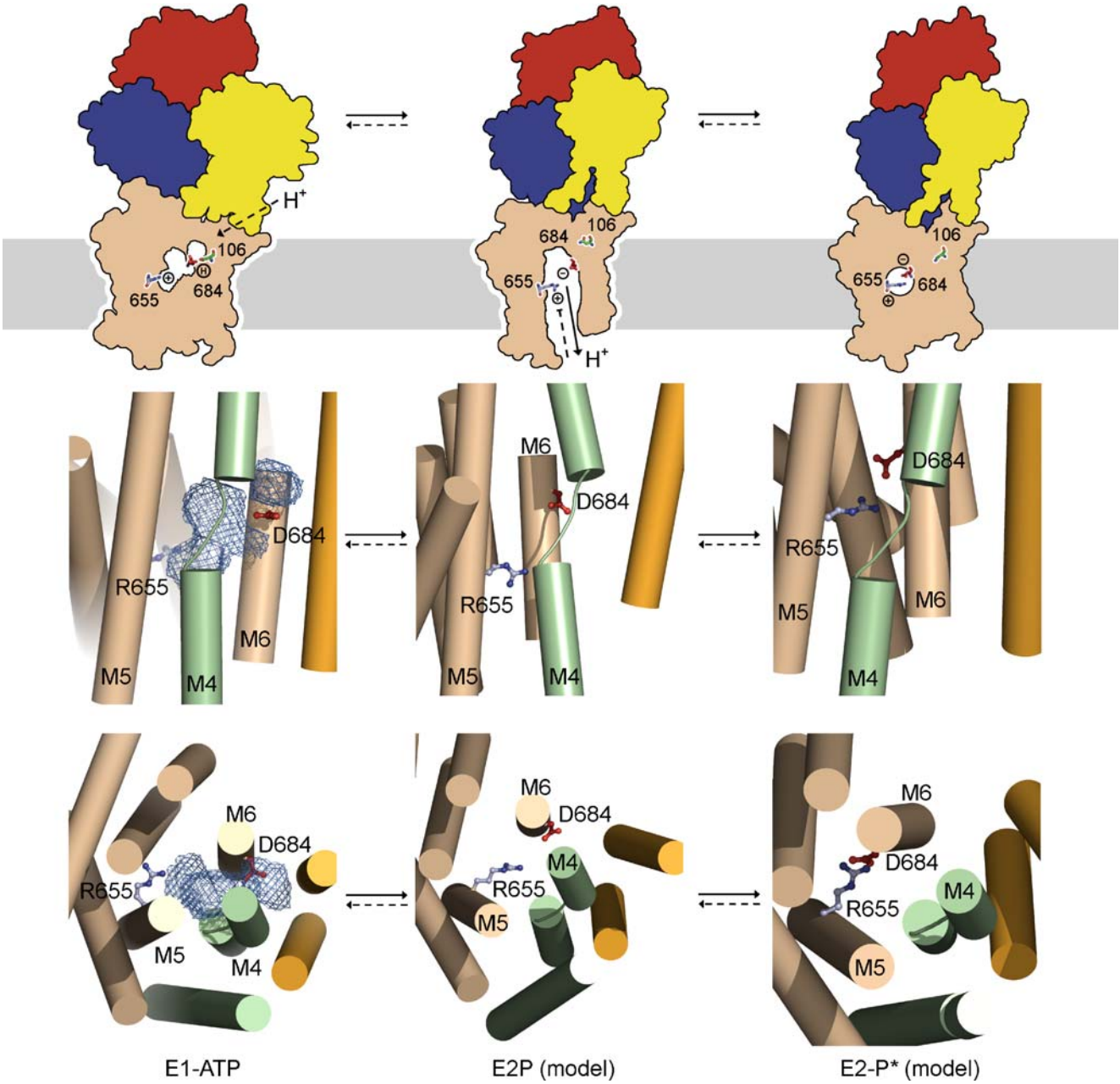

Fig. 3 Mechanism of proton transport by plasma membrane H^+-ATPase. *Upper row*, cartoon of the proton transport mechanism. *Middle row*, close up from the side. *Lower row*, close up from the top. The central cavity is marked with a *blue mesh*. Asp684 and Arg655 are marked in the ball and stick model in *red* and *blue*, respectively. Asp684 is the central proton donor/acceptor of the pump. Together with Arg655 it lines the centrally located water-filled cavity. In the E_1 conformation (*left*), hydrogen bonding between Asp684 and Asn106 gives preference to the protonated form of Asp684 (E_1-ATP structure). Conformational movements in the membrane region, coupled to E_1–E_2 transitions, result in the opening of the cavity towards the proton exit pathway (E_2P model) and interrupt hydrogen bonding between Asn106 and Asp684; this results in proton release from Asp684, now exposed to the extracellular environment (*middle*). Placement of Arg655 towards Asp684 at the exit channel also stimulates proton release from Asp684 and provides a positively charged plug in this area of the molecule that prevents extracellular protons from re-protonating Asp684 (*right*). At the same time, Arg655 functions as a built-in counter-ion that neutralizes the negative charge on Asp684 and promotes swift formation of the occluded E_2-P* transition state (E_2P* model), dephosphorylation and transition to the E_2 form. Although distantly placed from Asp684 in the determined E_1-AMPPCP structure, modeling of E_2 forms of the ATPase propose that Arg655 will approach Asp684 during the proton transfer event. The E_2 model forms of the H^+-ATPase were made by structural alignment of the E_1-AMPPCP H^+-ATPase structure with the E_2P structure of the Ca^{2+}-ATPase and the E_2-P* structure (E_2-occluded state of the pump) of the Ca^{2+}-ATPase [23]

allows a proton wire to assemble to the extracellular environment. Proton unloading from Asp684 will initiate at the cavity, which now forms the deep end of a long exit pathway. Arg655 is suggested to play a key role in proton release and proton pumping against high membrane potentials [25]. The positive charge of Arg655, now approaching Asp684, will favor proton release from Asp684 and at the same time inhibit reprotonation of Asp684 with an extracellular proton, which potentially could reverse pump action. In this way, Arg655 may constitute an important basis of the high-voltage performance of the proton pump. Another aspect of this is the proposed role of Arg655 as a counter-ion substrate 'mimic'. As soon as the exported proton leaves Asp684, a salt bridge may form with Arg655. This would lead to immediate closure of the pump to the E_2P-occluded state attained along with the assembly of the dephosphorylation site. Thus, the E_2P open state would be very short lived, minimizing exposure of Asp684 to the extracellular environment and providing a higher resistance to high membrane potentials (Fig. 3). In this state, Arg655 is predicted to be very close to Asp684 (Fig. 3).

Conserved charged amino acid residues at the bottom of the proton exit pathway may also support proton release, perhaps by providing the scaffold for binding of water molecules. A similar arrangement is seen in the proton release pathway of bacteriorhodopsin [13, 17]. The charged residues on the extracellular side of the pump seem to be divided in two layers: an outer positively charged layer and an inner negatively charged layer. It can be speculated that this creates a local environment favorable for proton unloading from the pump, to facilitate the generation of a high membrane potential. The outer layer could shield the pump from the high outer concentration of protons, while the inner layer could funnel proton movement away from the transmembrane proton binding site, Asp684, and the cavity.

Role for water in the transport of protons

The placement in the transmembrane domain of a large cavity, likely to be water-filled and with the central proton acceptor/donor exposed to its lumen, is a unique feature of the plasma membrane H^+-ATPase, differentiating its transport mechanism from other P-type ATPases whose structures are known (Ca^{2+}-ATPase and Na^+/K^+-ATPase). The relatively low resolution of the crystal structure does not allow individual water molecules to be identified, but the size of the cavity would allow for ten to 14 water molecules to be enclosed. The cavity may promote efficient proton transport in a least two ways. First, it may serve as a proton dump during catalysis and, second, it may serve to make the transport pathway of protons across the dielectric barrier of the membrane as short as possible. Water molecules in the cavity may be the initial acceptors for a proton released from Asp684. Such a scenario will lead to a delocalized hydrated proton in the water cluster. The merger of the cavity with the extracellular solution required for proton release would create a large aqueous vestibule traversing more than half of the membrane bilayer. The creation of such a large aqueous vestibule in essence means that the protein only needs to catalyze proton transport across a single essential proton acceptor/donor (Asp684). This elegant structural design strongly reduces the energy barrier required for the proton to cross the plasma membrane.

Conclusion: a unifying theme for proton transport

Like in other P-type ATPases, the transport mechanism of plasma membrane H^+-ATPases is best described by an alternating access mechanism. The central proton acceptor/donor is a single protonable aspartic acid residue (Asp684). The catalytic machinery is optimized to sustain efficient and regulated proton loading and unloading of this residue during conformational transitions with no associated proton short circuit taking place. The proton transport pathway going to and from Asp684 is likely to include charged and/or polar residues as well as centrally located water molecules. The fusion of the central-water-filled cavity with the outside of the cell serves to minimize the actual distance of proton transport.

The transport mechanism of plasma membrane H^+-ATPases bears striking resemblance to that of structurally distinct proteins as bacteriorhodopsin and the F_OF_1 ATP synthase (Fig. 2). In these pumps, the minimal pumping apparatus consists of a central proton acceptor/donor, a positively charged residue to control pK_a changes of the proton acceptor/donor, and bound water molecules to facilitate rapid proton transport. These are exactly analogous to those elements of proton transport that are proposed for the plasma membrane H^+-ATPases. Thus, it appears that the same basic machinery of proton transport has evolved convergently in as different protein structures as plasma membrane proton pumps, F_oF_1 ATPases and bacteriorhodopsin. This strongly suggests that the identified mechanistic elements of proton pumps could be universal for biological transmembrane proton-transporting systems.

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