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Review

Neurotransmitter release: the dark side of the vacuolar-H$^+$ATPase

N. Morel *

Laboratoire de Neurobiologie Cellulaire et Moléculaire, CNRS, 91198 Gif sur Yvette, France.

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Abstract

Vacuolar-H$^+$ATPase (V-ATPase) is a complex enzyme with numerous subunits organized in two domains. The membrane domain V0 contains a proteolipid hexameric ring that translocates protons when ATP is hydrolysed by the catalytic cytoplasmic sector (V1). In nerve terminals, V-ATPase generates an electrochemical proton gradient that is acid and positive inside synaptic vesicles. It is used by specific neurotransmitter-proton antiporters to accumulate neurotransmitters inside their storage organelles. During synaptic activity, neurotransmitters are released from synaptic vesicles docked at specialized portions of the presynaptic plasma membrane, the active zones. A fusion pore opens that allows the neurotransmitter to be released from the synaptic vesicle lumen into the synaptic cleft. We briefly review experimental data suggesting that the membrane domain of V-ATPase could be such a fusion pore.We also discuss the functional implications for quantal neurotransmitter release of the sequential use of the same V-ATPase membrane domain in two different events, neurotransmitter accumulation in synaptic vesicles first, and then release from these organelles during synaptic activity.

Keywords: Fusion pore; Exocytosis; Synaptic vesicle; Membrane fusion; Proteolipid

Neurotransmitters are synthesized in nerve terminals, far away from the cell body. They are stored within synaptic vesicles and released in the synaptic cleft, close to their post-synaptic receptors. Neurotransmitter release occurs at specialized areas of the nerve terminal membrane, the active zones. Under resting conditions, a subpopulation of synaptic vesicles is docked to the active zone membrane (Couteaux and Pécot-Dechavassine, 1974; Harlow et al, 2001), close to voltage-gated calcium channels (Robitaille et al, 1990). During synaptic activity, depolarisation of the nerve terminal by the action potential activates these calcium channels, triggering a calcium influx through the presynaptic membrane. The cytosolic calcium concentration raises abruptly in nerve terminals, reaching transiently the submillimolar range (200 μM and more) in microdomains underlying the active zones (Llinas et al, 1992). This triggers neurotransmitter release, that occurs less than 200 μseconds after calcium channel activation (Llinas et al, 1981). These very rapid presynaptic events appear to very much resemble much slower membrane traffic and fusion processes, involving proteins that are conserved from yeast to man (Jahn and Südhof, 1999; Wickner and Haas, 2000). Vacuolar proton ATPase (V-ATPase) is one of these proteins. It is required to concentrate neurotransmitters in synaptic vesicles and could also be important for membrane fusion.

1. V-ATPase and neurotransmitter storage in synaptic vesicles

V-ATPases translocate protons across the membrane of various intracellular acidic organelles (lysosomes, endosomes, *trans*-Golgi cisternae, secretory granules, etc...) (Nelson and Harvey, 1999; Nishi and Forgac, 2002). Acidification of these organelles is required for many cellular processes involving intracellular membrane traffic (maturation and processing of proteins, receptor-mediated endocytosis, proton-coupled transport of small molecules, etc...). V-ATPases are large multimeric enzymes, made of several different subunits organized in two domains (Fig. 1). The catalytic V1 domain (500 kDa) is composed of eight different subunits (A-H) with three copies of the ATP-binding subunits A and B. The membrane domain V0 (250 kDa) contains six proteolipid subunits (5 c-subunits and 1 c”-subunit) and single copies of subunits a and d (Nelson & Harvey, 1999; Nishi & Forgac, 2002). The proteolipid subunits constitute a hydrophobic ring involved in proton transport. ATP hydrolysis by the three A subunits of V1, in the cytoplasm, induces

* Corresponding author.
Tel.: (33) 01 69 82 36 47; fax : (33) 01 69 82 94 66.
E-mail address: nicolas.morel@nbcm.cnrs-gif.fr (N. Morel).

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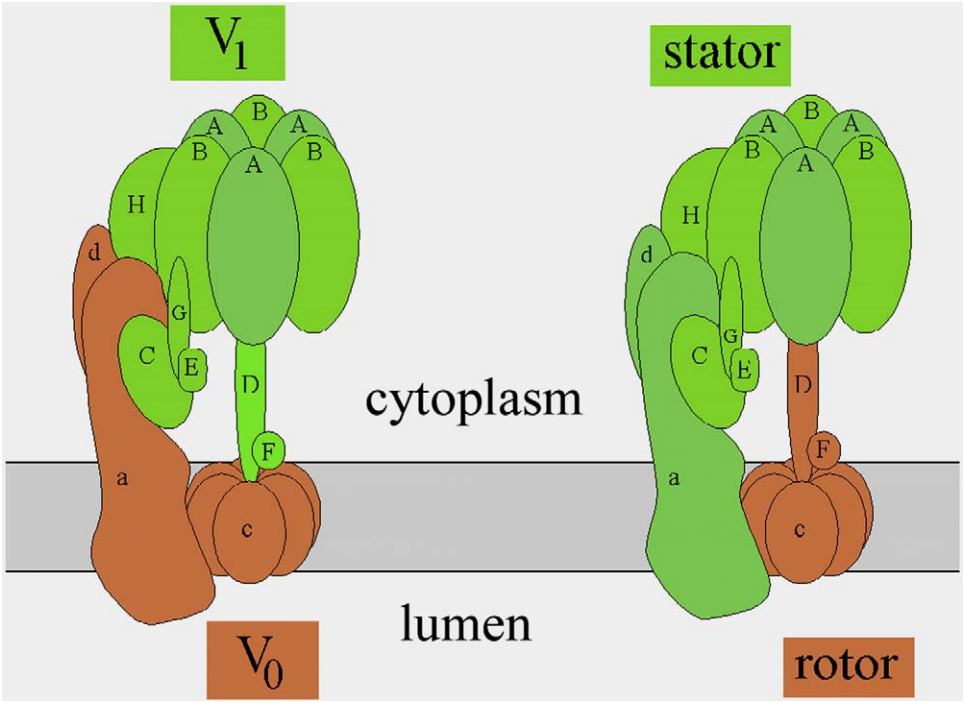

Fig. 1. Functional organization of V-ATPase.
V-ATPase is composed of numerous subunits organized in two domains. The cytoplasmic V1 domain contains 8 different subunits (A to H), with 3 copies of the ATP binding subunits A and B. The membrane V0 domain is made of an hexameric ring of proteolipid subunits (4-5 c-subunits and 1 c"-subunit) and single copies of subunits a and d. ATP hydrolysis by the 3 subunits A induces the rotation of several subunits of both the V1 and V0 domains (rotor): a stalk made of subunits D and F and, in the membrane, the proteolipid ring. Subunits which do not move constitute the stator. Rotation of the rotor induces the translocation of protons that are bound at the periphery of the proteolipid hexamer, at the lipid interface. The V1 domain reversibly dissociates, in physiological conditions, to regulate V-ATPase activity.

the rotation in the membrane of this proteolipidic ring (Fig. 1) and the translocation of protons that are bound at its periphery (Nelson and Harvey, 1999). Subunit a of the V0 membrane domain is an integral membrane protein, containing multiple transmembrane helices located in its C-terminal half, with a large amino-terminal sector in the cytoplasm (Leng et al, 1999). Multiple isoforms of subunit a have been identified, with specific organelle or cellular distributions. In mice, four different isoforms (a1 to a4), encoded by different genes with tissue-specific expression, have been described (Toyomura et al, 2000; Nishi and Forgac, 2000; Oka et al, 2001). The diversity of subunit a isoforms is not only important for tissue specificity and targeting to different membrane compartments, it could also result in the generation of V-ATPases with different functional properties (Kawasaki-Nishi et al, 2001). In contrast to subunit a, in vertebrates, other subunits of the membrane domain of V-ATPase are encoded by single genes (Nishi and Forgac, 2002).

In neurons, V-ATPases are also present in the membrane of synaptic vesicles, the neurotransmitter storage organelles, (Yamagata & Parsons, 1989). The large electrochemical H^+ gradient generated by this enzyme, pH 5.2-5.5 inside the synaptic vesicles (Michaelson & Angel, 1980; Füldner & Stadler, 1982), is used by specific vesicular transporters to accumulate the neurotransmitter. Neurotransmitter concentrations can be quite high within synaptic vesicles, up to 500 mM for *Torpedo* electric organ cholinergic synaptic vesicles (Ohsawa et al, 1979). Using patch-amperometry on single chromaffin cells, Albillos and coworkers (1997) found that all granules that are exocytosed contain approximately the same concentration of catecholamines (0.7 M), regardless of granule size. Within a nerve terminal, synaptic vesicles distribute between a large reserve pool, and a small (5-10 %) population of active vesicles. These active vesicles, probably docked at the active zones, are preferentially used to release the transmitter during synaptic activity (Suskiw et al, 1978; Koenig and Ikeda, 1999; Südhof, 2000). When emptied, these vesicles refill from the newly synthesized cytoplasmic neurotransmitter and can be used several times without mixing with the reserve population (Suskiw et al, 1978; Südhof, 2000; Pyle et al, 2000). Neurotransmitter release is quantal and the size of released quanta is constant in physiological conditions (Van der Kloot and Molgo, 1994). If the quantum corresponds to the synaptic vesicle neurotransmitter content, one has to assume that the release machinery is able to select, among all synaptic vesicles accumulated at the active zone, those that are filled with the neurotransmitter or able to ignore synaptic vesicles that are in the act of replenishing.

2. V-ATPase and fusion pore

The functional importance of SNARE proteins for synaptic vesicle docking and synaptic transmission is well docu-

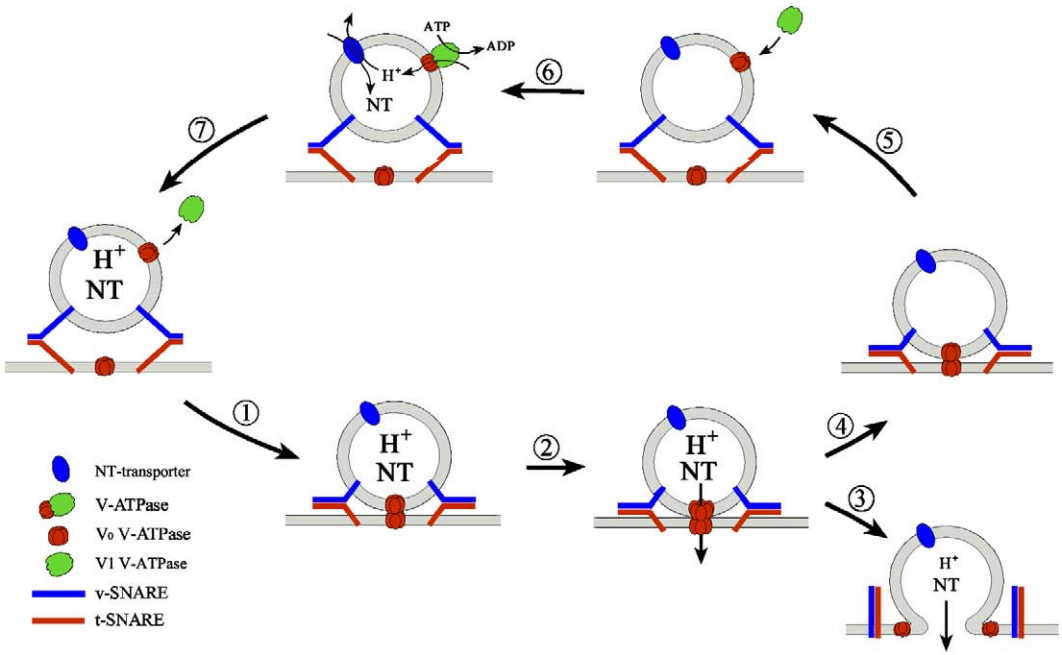

Fig. 2. V-ATPase and neurotransmitter release.

Synaptic vesicles are docked at the presynaptic plasma membrane (1) by SNARE complexes, associating the synaptic vesicle protein VAMP-2 (v-SNARE) and syntaxin-1 and SNAP-25, two presynaptic membrane proteins (t-SNAREs). A fusion pore forms and opens after activation by calcium (2), allowing the release of small soluble molecules from the synaptic vesicle in the synaptic cleft. The fusion pore could be a dimer of two opposing V-ATPase membrane domains (V0 V-ATPase). The pore either expands leading to membrane fusion (3), and the full release of synaptic vesicle contents, regardless of their size. Alternatively, the pore closes after some delay (4), its transient opening being sufficient to release the synaptic vesicle neurotransmitter (NT). V-ATPase activates (5) and accumulates protons allowing neurotransmitter uptake (6). When neurotransmitter concentration is achieved, the vesicular transporter no longer dissipates the electrochemical gradient, which reaches maximal values. V-ATPase activity is turned off by dissociation of the catalytic sector V1 (7). The V0 domain is now unmasked and ready to form the V0-V0 fusion pore. This would explain how the release machinery knows that synaptic vesicles are filled with neurotransmitter and ready for release.

mented (Jahn and Südhof, 1999; Bruns and Jahn, 2002). The synaptic vesicle protein VAMP/synaptobrevin-2 forms with two proteins of the presynaptic plasma membrane (syntaxin-1 and SNAP-25) a very stable *trans*-SNARE complex that brings in close apposition the synaptic vesicle and presynaptic plasma membranes (Fig. 2-1). Cytosolic calcium induces the opening of a fusion pore of low selectivity and large conductance that spans the two membranes (Breckenridge and Almers, 1987 ; Alvarez de Toledo et al, 1993) . This allows the release in the synaptic cleft of small solutes concentrated in synaptic vesicles (Fig. 2-2). The fusion pore can then expand, leading to membrane fusion and exocytosis of all synaptic vesicle contents (Fig. 2-3), or the pore can close after some delay (Fig. 2-4). High calcium concentrations favor transient pore opening events (Alés et al, 1999). In nerve terminals, such conditions only prevail at the active zone microdomains, during synaptic activity. In fast operating synapses, it is therefore likely that neurotransmitters are released through transiently opened fusion pores, without collapse of synaptic vesicles into the presynaptic plasma membrane. The molecular nature of these fusion pores, purely made of lipids or lined by proteins, is still a matter of debate (Bruns and Jahn, 2002; Mayer, 2001; Zimmerberg, 2001).

Several experimental lines of evidence suggest that the membrane sector of V-ATPase or its proteolipid ring could be such a fusion pore. The first series of experiments concerns functional reconstitution of purified V-ATPase proteolipids in artificial membranes. When incorporated in the membrane of liposomes filled with acetylcholine, the V-ATPase c-subunit from *Torpedo* nerve terminals form homo-oligomers (mediatophores) that are able to release the neurotransmitter upon calcium activation (Israël et al, 1986; Morel et al, 2001). Similarly, V-ATPase proteolipids purified from yeast vacuoles form pores in artificial membranes, that open in the presence of calcium and calmodulin (Peters et al, 2001). The second sort of experiment relies on cell transfections with plasmids encoding the V-ATPase c-subunit. Transfected neuroblastoma cells overexpressing the *Torpedo* V-ATPase c-subunit gain a calcium-dependent acetylcholine release mechanism with quantal properties (Falk-Vairant et al, 1996; Bloc et al, 1999). Finally, experiments on homotypic vacuolar fusion in yeast showed that, after *trans*-SNARE complex formation, the vacuole docking step is followed by a calcium/calmodulin-dependent step (Wickner and Haas, 2000). During this final step leading to vacuole fusion, calmodulin interacts with the V0 membrane domain of V-ATPase (Peters et al, 2001). A V0-V0 *trans*-complex forms by pairing of membrane sectors of V-ATPases in the contacting vacuoles, that constitutes a pore spanning the two apposed membranes (Peters et al, 2001). The hydrophobicity of the proteolipids lining the pore would allow its radial

expansion by interposition of membrane lipids during membrane fusion (Zimmerberg, 2001).

The membrane domain of V-ATPase could therefore be the fusion pore involved in membrane fusion (Bruns and Jahn, 2002; Mayer, 2001; Zimmerberg, 2001) and neurotransmitter release (Morel et al, 2001). The same structure would play two completely different roles, translocate protons when associated with the catalytic V1 domain of V-ATPase and constitute a fusion pore when interacting with another V0 V-ATPase sector. Considering the high conservation of the V-ATPase membrane domain subunits, from yeast to vertebrates (Nelson and Harvey, 1999; Nishi and Forgac, 2002), some functional constraints must have opposed to a divergent specialization during evolution of the V0 domains that transport protons and those involved in membrane fusion.

3. V-ATPase as a sensor of synaptic vesicle content ?

Use of the same synaptic vesicle V0 domain of V-ATPase, first associated to the catalytic sector V1 to translocate protons and, then, in association with a V0 domain of the presynaptic plasma membrane in the fusion pore, could result in an unexpected third function for V-ATPase. Active V0-V1 V-ATPase generates an electrochemical proton gradient that is used by specific transporters to accumulate a given neurotransmitter in the synaptic vesicle (Fig. 2-6). When neurotransmitter refilling is completed, the vesicular transporter no longer dissipate the electrochemical proton gradient, which can then reach maximal values. We suggest that this leads to V-ATPase dissociation, and release of the V1 domain in the cytosol (Fig. 2-7). The reversible dissociation of V1 has been shown to occur in physiological conditions as a means to regulate V-ATPase activity (Kane, 1995). The V0 subunit a possesses conserved aminoacids located in the vesicular lumen that control assembly of the cytoplasmic V1 domain to the V0 sector (Leng et al, 1999). Dissociation of V-ATPase unmasks the V0 domain only when the proton gradient, and the vesicle neurotransmitter content, is maximal. This V0 domain is now ready to interact with another V0 domain, present in the presynaptic plasma membrane (Morel et al, 2001), and form a new fusion pore (Fig. 2-1). Such a mechanism would explain why only fully filled vesicles are used to release the neurotransmitter, explaining the quantal nature of synaptic transmission.

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