Chapter 4
Calcium Controls the P2-ATPase Mediated Homeostasis: Essential Role of NaAF

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Abstract This chapter reveals a new unique story of Ca-signaling in upholding cellular homeostasis. The cytosolic activator (regulatory) protein, NaAF (of 170 kDa mass), for the ubiquitous P2-ATPase (and 80 kDa HAF solely for the gastric H/K-ATPase) is essential for P2-ATPase function. The NaAF and HAF function as the allosteric operator-cum gate-keeper of the dual channel P2-ATPase system (where mirror-image orientation of the two α-subunits serves as the membrane-embedded in-and-out gates) for simultaneous transport of two ions. The entire cyclic operation is in turn fine-tuned by local Ca (μM) as top (allosteric) controller of the P2-ATPase to maintain homeostasis. Thus at lower range Ca (<2) stimulates, but at higher range (>2) Ca abruptly inhibits the HAF-stimulated H/K-ATPase abolishing it at 4 μM Ca. At this point the (K ± HAF)-independent basal (Mg-dependent) activity of the H/K-ATPase acts as a provisional Ca-ATPase pump in an altered state to remove excess Ca, thus resuming the initial Ca-activated HAF-regulated state of a new cycle. Identical Ca-signaling operations also control the universal NaAF-regulated Na/K-ATPase system.

Keywords P2-ATPase (s) • Cytosolic activator protein • Allosteric regulation • Ca-signaling • Homeostasis

1 Introduction

Michael Berridge [1] has recently reviewed the details of many universal Ca-signaling processes, which he initially mapped over a decade ago [2]. The present work describes the delicate control mechanism of active ion transport by μM Ca published in the 1980s based on a new dual-topology model for the P-2 ATPase

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system, since the existing Post-Albers single topology scheme (for sequential ion transport) reining the P2-ATPase field was inadequate to explain this. The new dual-topology P2-ATPase model first introduced in 1986 [3] accommodates all the limitations of PA scheme and also demonstrates the universality of active ion transport by cytosolic regulatory protein and μM Ca mentioned above.

Our bodily cells have built-in ion gradient; the intracellular concentrations of different metal ions (such as, Na, K, and Ca) are very different from those present in the blood transporting them to tissues all through the body; the circulating blood of an individual maintains a fixed concentration (~4 mM) of Na (~140 mM), K (~4 mM), and Ca (~5 mM) compared to the cell interior having Na (~10 mM), K (~140 mM), and Ca (in varying μM range) respectively. So, it is essential for a living cell to maintain the ion gradient (homeostasis) which is at a constant flux with the extracellular environment for carrying out numerous essential functions. As a result, the cells have built-in 24/7 ion-pumps, such as H/K-ATPase, Na/K-ATPase (Na-pump), and Ca-ATPase (Ca-pump), called the P2-ATPase system, on the plasma membrane to pump out the excess metal ions maintaining homeostasis. The cell Ca is used for many important tasks like membrane fusion, turning on and off the key intercellular processes as a final regulatory and other cellular signaling [4, 5].

The plasma membrane Ca-ATPase (PMCA) pumping out excess Ca is indeed a provisional operation of the basic P-2 ATPase systems [5] like the gastric H, K-ATPase [6] and the Na, K-ATPase, working in altered state as a Ca-ATPase based on local need of the host cell (see below). After the job is done the Ca-pump switch back to the former state pumping H or Na as the case may be. The entire operation of the provisional Ca-Pump depends on the nature of the cytosolic regulator (discussed below) running the original pump (H- or Na-Pump). This chapter deals with the HAF-dependent Proton-Pump and Na-Pump belonging to the apical (secretary) membrane (APM) and basolateral membrane (BLM) of parietal cell, and then extending further to the analogous nonparietal Na-Pump as needed for clarification. The details on Ca-signaling will follow.

Let me introduce at first the new dual-topology model of gastric proton-pump giving a unified view of the HAF-regulated pumping of H/K, Ca/H and Na/K, Ca/K across the APM and BLM where the signaling role of Ca is revealed under different conditions of local pH and Ca.

2 The Dual-Topology H, K-ATPase System Is a General Model for the Simultaneous Bidirectional Transport of H/K, Na/K, and Ca/H Across the Apical Plasma Membrane

The construction of the dual-topology model is based on hard data on the orientation of critical ligand sites within a functional H, K-ATPase complex associated with tightly sealed gastric microsomal vesicles of uniform orientation that are capable of ATP-dependent accumulation of H in exchange for K [3]. The dual-topology H,

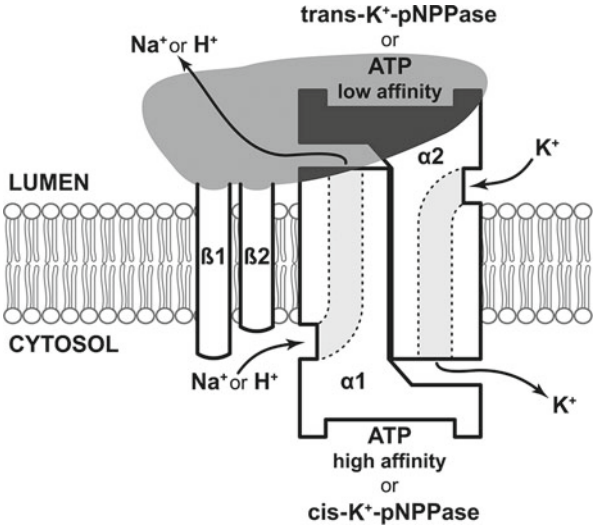

Fig. 4.1 The dual-topology Na (H), K-ATPase showing bilayer orientation of α and β subunits with related ion channels. Two identical subunits, α1 and α2 (not isomers), are shown in mirror images across the membrane with embedded ion channels in contact, and are held with two closely associated β-subunits facing the lumen. The ATP hydrolytic site (separate from the *cis*-pNPPase site) on the α1, and *trans*-cytosolic non-hydrolysable ATP-binding site and the corresponding *trans*-pNPPase site on the α2 are shown. Besides ATP-binding the intimate association among α2, β1 and β2 on the cell exterior is expected to modulate the ATPase function in various ways including the reception of extracellular signals. The high-affinity K^+ site for ATPase stimulation is located across the bilayer on α2 and the corresponding high-affinity H^+ or Na^+ site is on the cytosolic side of the α1 enabling access gating. The low-affinity K binding sites responsible for releasing the transported ions are present at or near the exit end of the related ion channel on each side of the bilayer. Under appropriate conditions of local ionic milieu, the versatile P-2 ATPase pump transports Na/K, H/K, and Ca/H in altered states [3, 4]

K-ATPase described recently [3, 4] is a paradigm shift from the 50-year-old single topology model dominating the P2-ATPase field. We observed earlier that the K-stimulated para nitro phenyl phosphatase (K-pNPPase), co-purified with the gastric H, K-ATPase, was not a partial reaction of the gastric ATPase [3, 4] as postulated in the single topology Post-Albers scheme. As the name "dual topology" implies, new model of H, K-ATPase has two 100 kDa α-subunits in mirror-image orientation across the membrane, in contrast to existing "single topology" one having only one α-subunit facing the cytosol (Fig. 4.1). The new model has two low-affinity K-para nitro phenyl phosphatase (K-pNPPase) sites (one on each α-subunit) regulating simultaneous transport of H/K across (Fig. 4.1). Generality of the dual-topology model was revealed by the identical orientation of K-pNPPase across the isolated surface epithelial cell outer membrane capable of dose-dependent ^{86}Rb uptake inhibitable by ouabain [7].

The HAF is intimately involved in pump operation (Fig. 4.1) from the beginning of the pumping process, such as binding of ATP to the ATPase catalytic site, up to

the end of pumping cycle by carrying out the bidirectional transport of H and K across, and then beginning a new phase. In fact, during its allosteric pumping execution the HAF creates the high-affinity K-effector site of the dual-topology H, K-ATPase (facing the lumen) for exchange with cytosolic H. It must be noted that without the availability of luminal high-affinity K site the pump cannot function. Such crucial role of HAF was confirmed by the use of mono-specific anti-HAF antibody that blocks the HAF-stimulated H, K-ATPase activity in vitro, as well as blocked the production of protons in vivo when inserted into digitonin-permeabilized rabbit gastric glands (Zenodo, DOI: 10.5281/zenodo.7093).

The adjoined α subunits with embedded ion channels in mirror-image orientation (Fig. 4.1) are believed to oscillate laterally within the plane of the membrane during the pump operation [4]. Oscillation of the ion channels is initiated by cytosolic activator (HAF)-dependent activation of the enzyme (E) forming E*.ATP at the catalytic α_1 site with simultaneous binding of high-affinity H (Na) to a nearby site in the cytosol (see below). Binding of H (Na) induces domain-domain interaction between the membrane-embedded helixes of α_1 and α_2 with the resultant binding of high-affinity K at the *trans*-cytosolic α_2 site. This H (cytosolic) and K (luminal) bound transitional complex (E*ATP.H.K) spontaneously hydrolyze the ATP helping the H, K-ATPase molecule return to its original configuration (E) following a harmonious shift of the adjacent ion channels back to its initial state. The entire process creates a peristaltic movement of both ion channels for the bidirectional transport of H and K across. Please note that in the case of nongastric tissue, the P2-ATPase pump transports Na and K mediated by an analogous regulatory protein, NaAF (of 170 kDa mass), universally present in all bodily cells except gastric parietal cells. The detail on HAF regulation of the proton-pump is discussed in the following section.

3 Allosteric Regulation of the Gastric ATPase System by Its Cytosolic 80 kDa HAF and Ca (μM)

In the active state, the 80 kDa HAF is a dimer of two identical 40 kDa subunits; the monomers are totally inactive [3]. The dramatic nature of HAF activation of the gastric H, K-ATPase system is shown in Fig. 4.2. During the rapid allosteric activation of the pump (Hill coefficient = 4.5), eight to ten molecules of HAF interact cooperatively with each H, K-ATPase pump unit for optimal stimulation. The negatively charged HAF (80 kDa mass) consisting of 39 % nonpolar, 33 % polar uncharged, 5 % positively charged, and 20 % negatively charged amino acids [8] is suited for domain-domain interaction amongst themselves as well as interfacing the neighboring ATPase catalytic domain. Previous reconstitution studies on gastric microsomal H, K-ATPase following inactivation by mild perturbation of annular lipids [9, 10] suggest the HAF molecules to be loosely anchored to some phosphatidyl choline (PC) having distinct fatty acid (FA) compositions (80 % saturated, 20 % mono- and di-unsaturated lacking in polyunsaturated FA). It appears that the

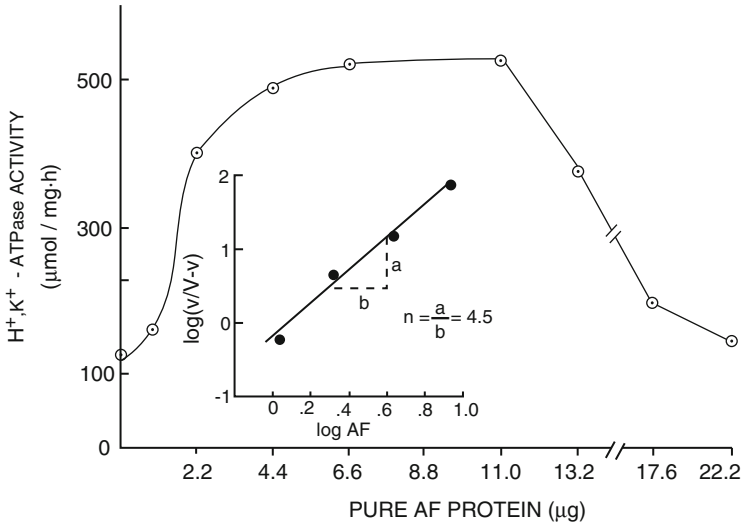

Fig. 4.2 Effects of increasing concentrations of pure HAF (described as AF) on the APM-associated pig gastric H, K-ATPase activity. 4 µg of the pig H, K-ATPase and indicated amount of the pure HAF were preincubated for 10 min at 37 °C in Pipes buffer (pH 6.8) and assayed [6]. Data are the average of triplicates. The *inset* shows the Hill plot. This figure shows highest cooperative activation with 11 µg HAF and 4 µg nearly homogenous ATPase. Assuming the MW of gastric H, K-ATPase to be 320,000 we estimated that each nmol of H, K-ATPase binds 10 nmol of HAF. Note the dramatic downregulation with further increase in HAF level. It is noteworthy in this connection that the activity of K-pNPPase is also significantly increased without any alteration of the low-affinity K site [Taken from Ref. 8]

negatively charged HAF molecules are anchored to the cytosol-facing head group of PC at the annular zone by entropy-driven process discussed below.

Inspection of the various thermodynamic parameters of the ATPase activation process reveals that the HAF activation of the H, K-ATPase is entropy driven [8]. About eight to ten molecules of HAF as a single unit boost the ability of each H, K-ATPase to generate optimal transition state (E*.ATP) complex by cooperative interaction in their cytosolic ambience (37 °C) by increasing the entropy of activation ($\Delta S‡$) of the system, thereby causing the simultaneous allosteric binding of high-affinity Na (or H) and high-affinity K in the lumen across. This is how the HAF appears to initiate the lateral movement of the transmembrane helixes of mirror-image (α1α2) orientation for simultaneous binding and transport of both ions during ATP hydrolysis [8], then shifting back immediately to the original state (E) to begin the a new cycle with fresh formation of E*.ATP.

However, following the optimal activation of H, K-ATPase at 1:10 (mentioned above) the K-stimulated activity is drastically reduced being eliminated at 1:14 (ATPase to HAF) reaching the basal (Mg) state. This might be the way proton-pumps enjoy momentary rest prior to returning to the intracellular tubulovesicular (TV) storage pool staying fused to it.

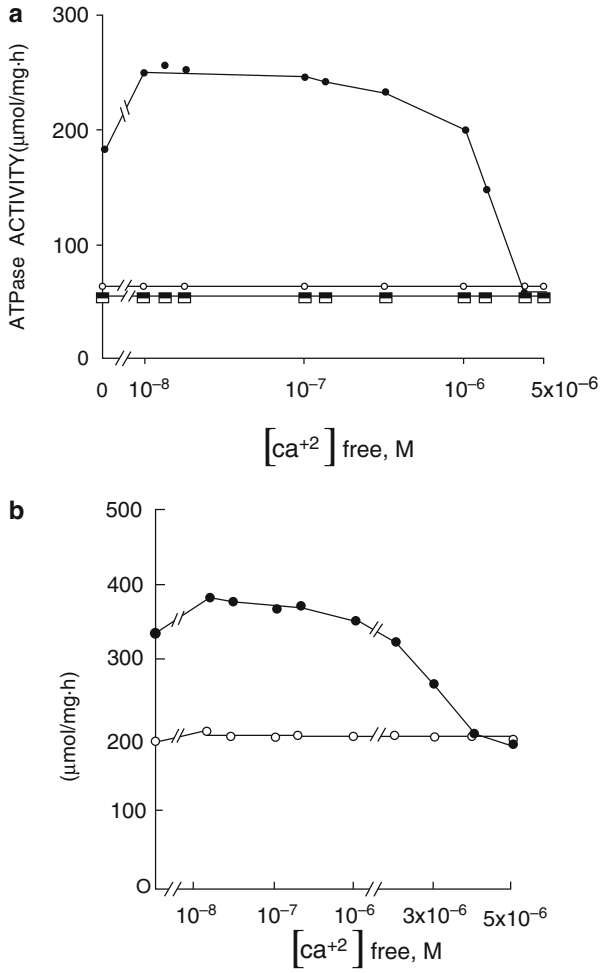

Fig. 4.3 (**a**) Effects of µM Ca on HAF-dependent H, K-ATPase activity associated with low-density APM. Without HAF without K (*open square*) and with K (*closed square*); with HAF without K (*open circle*) and with K (*closed circle*). (**b**) Effects of µM Ca on HAF-dependent H, K-ATPase activity associated with high-density BLM in the absence (*open circle*) and presence (*closed circle*) of the HAF. Note the similar stimulation followed by inhibition in both **a** and **b** with increasing µM Ca. Note that similar to APM above only the HAF-dependent activity associated with BLM is abolished. Data taken from Ray et al. [11]

The critical role of µM Ca in the regulation of the HAF-stimulated gastric H, K-ATPase system associated with the APM and BLM is shown in Fig. 4.3. The dramatic sensitivity of the HAF-regulated gastric H, K-ATPase system to physiological Ca level is obvious from Fig. 4.3 [11].

The HAF-stimulated H, K-ATPase activity under steady-state condition shows additional allosteric stimulation at very low level of Ca (<2 µM); beyond this the

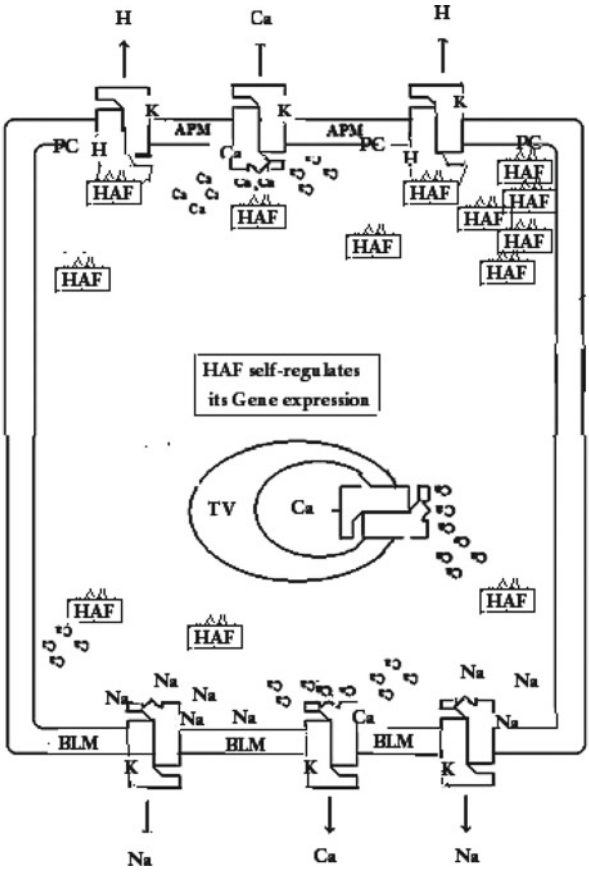

Fig. 4.4 Critical interplay of Calcium in the HAF regulation of the gastric H, K-ATPase Pump (pumping H against a millionfold gradient compared to intracellular pH) at the APM showing oscillation of the Pump between H- and Ca-transporting modes depending on the local Ca level. The APM pump works well between 1 and 2 μM Ca but abruptly stops between 2 and 4 μM Ca. In a similar fashion the H-pump at the basolateral membrane (BLM) works as a Na-Pump and a Ca-pump based on local levels of high Na, high Ca, and higher pH (bicarbonate tide) due to proximity to blood supply. This figure also shows clustering of the HAF molecules bound to the APM-associated polar group of phosphatidyl choline that facilitates allosteric activation (E*.ATP) of the proton-pump by forming domain-domain interaction neighboring HAF mentioned earlier. Note that the annular PC molecules providing the proper fluid environment for the H, K-ATPase function have been identified to consist of saturated (16:0 and 18:0) and unsaturated (18:1 and 18:2) fatty acids lacking in 20:4 [9, 10]

HAF-stimulated H, K-ATPase is drastically downregulated, coming to a halt at 4 μM. At this point the H-pump acts as a provisional Ca-pump to pump out excess Ca, thus resuming the H-pump activity (Fig. 4.4). Hence, higher Ca (2–4 μM) acts as a feedback control switch for turning off/on the HAF-operated H-pump as the top regulator.

4 Critical Interplay of Calcium in the HAF-Regulated H, K-ATPase and Na, K-ATPase Pumps in Gastric Parietal Cell

For each mole of HCl secreted by the parietal cell, one mole of ATP is consumed. Hence, the mitochondria-enriched parietal cells have a constant supply of metabolic substrates, which are in turn co-transported with Na across the BLM. As a result, for each mole of H transported across the APM, an equivalent amount of Na enters into the cell across the BLM border, which is promptly pumped out again to maintain ionic homeostasis. So, for each mole of HCl secreted the parietal cells spend two moles of ATP and produce two moles of HCO_3 (byproduct) which is promptly transported out (by Cl/HCO_3 exchanger) in exchange for Cl across the BLM to maintain pH homeostasis. These activities together with other major membrane processes undergoing gastric acid secretion, such as consistent trafficking of the H, K-ATPase molecule back and forth between the APM and intracellular reserve as tubulovesicles (TV), make the parietal cell membranes most active next to brain cells of the human body. The TV acting as the intracellular reservoir of H-pumps saves substantial energy for the cells. As shown in Fig. 4.4, the cytosolic HAF appropriately controls both the proton-pump on APM and the BLM (basolateral) Na-pump of the gastric P2-ATPase system acting alternatively as a provisional Ca-pump to maintain homeostasis. Please note that similar to parietal cell (Fig. 4.4, below) the NaAF-regulated ubiquitous Na, K-ATPase system belonging to all other tissues shows similar altered function as a provisional Ca-pump [1–3].

It is clear from the preceding information (Figs. 4.1, 4.2, 4.3, and 4.4) that the allosteric operation of the gastric H, K-ATPase system, pumping H, K, Na and Ca, is totally dependent on the cytosolic HAF acting as the operator of the bidirectional H/K-ATPase pump. During the pump operation (Figs. 4.1 and 4.4), the HAF helps to bind concomitantly the cytosolic H and luminal K (both with high affinity) for the simultaneous transport across the APM in opposite direction. Same thing happens with the H, K-ATPase at the BLM where in the alkaline environment the H/K--ATPase pump acts as the Na/K-pump; and in both situations the HAF acts as a faithful gate-keeper of ions for the parietal cell P2-ATPase system [5]. Thus, to carry out the dynamic functions of the H, K-ATPase the HAF appears to function as an operator-cum gate-keeper for managing the heavy ion-traffic across the plasma membrane gates, where the dual-topology ($\alpha^2\beta^2$-isoform) setting (Fig. 4.1) only serves as double gates for the passage of ions. In an analogous manner, the NaAF acts as the operator-cum gate-keeper of the ubiquitous Na, K-ATPase system.

5 Extracellular and Intracellular Ca-Environments of the Parietal Cell Under the Resting and Hormone-Stimulated Conditions

Under resting condition, the luminal or secretary environment of the parietal cell is generally smooth with neutral or slightly alkaline pH. In the cytosol, there are numerous mitochondria and tubulovesicular (TV) membranes (highly enriched in gastric H, K-ATPase activity) acting as a proton-pump reserve [12]. Following stimulation of acid secretion by the secretagogues, a spectacular transformation takes place within few minutes. Numerous TV membranes migrate towards the apical (secretary) plasma membrane (APM) causing fusion of proton-pumps with the resultant appearance of numerous secretary cannelicular projections into the lumen. During peak secretion the parietal cells secrete acid against a concentration gradient of over a millionfold (luminal pH nearing 0.1).

5.1 The State of [Ca] Under Resting Condition

Waves of Ca arising from the BLM locale have been reported in parietal cells [13, 14]. Most likely Ca enters the parietal cells through BLM via its InsP3R (receptor operated Ca-channel) creating Ca-waves along the intracellular tubulovesicles, TV (storage for proton-pumps), reaching the APM site in mild waveforms. In resting cell Ca remains pretty active in the mid-cell region TV pool in the following way. The Proton-Pumps (H, K-ATPase molecules) associated with this inside-out TV (vesicles) have the ATP hydrolytic (catalytic) site facing cytosol surrounded by the HAF pool. The presence of high Ca-waves (2–4 μM) in that mid-cell region will prevent the HAF from interacting with the ATPase site, forcing them to pump Ca (Fig. 4.4) into the vesicle interior for storing (as Ca-sink), thus keeping the proton-pumps truly at rest without wasting further energy. Upon receiving the signal for acid secretion, the Ca-loaded TV migrates towards the APM and the BLM for transferring the pumps to these sites. The mitochondria-loaded parietal cells would also store Ca in the mitochondrial matrix to activate a key Krebs cycle enzyme, thus meeting the ATP demands during acid secretion.

5.2 The State of [Ca] Under Stimulated Conditions

Following stimulation of the parietal cells Ca would be needed for the organized cytoskeletal movement of the TV towards the secretary APM site (of lighter buoyant density compared to TV) for the incorporation of new Proton-Pumps. As for the BLM site, however, there seems to be a different kind of mechanism at work,

since no such organized movement of TV towards BLM has ever been reported. In this case the Ca-loaded TV vesicles surrounded by the HAF pool seem to move towards the BLM environment by virtue of having identical ($d = 1.115$) buoyant densities [11]. In this environment TV easily mingles with the BLM by the whirlpool movement created by the highly active Na/K-pump, thus bringing them close together for the transfer of new pump molecules by Ca-mediated fusion. Such fusion between the Ca-loaded TV and high Ca BLM locality will be spontaneous due to identical lipid make up [9] of the ion-pumps.

6 Signaling Roles of Ca Under the Resting and Stimulated States of Parietal Cell

The specialized plasma membrane microdomains, "Caveolae or lipid rafts," made up of Sph and cholesterol have been implicated in Ca mobilization [15]. The lipid rafts would control the polar region of the parietal cell and regulate various Ca-signaling events. The critical constituent of lipid rafts, Sph, is very high in APM (66 %) and TV/BLM (59 %) consisting entirely of saturated fatty acid (SFA), such as the unique 14:0 (35.7 %), along with 16:0 and 18:0 with traces of unsaturated FA [11, 16]. Following stimulation, massive movement of TV towards the secretary cannelicular region of APM takes place, causing extensive membrane fusion, where the caveolae (by virtue of its cytoskeleton dynamics) would be acting as scaffolds for Ca ion channels, thus connecting the intracellular stimuli to extracellular milieu of the cell. Also, during the fusion of approaching TV with the APM (following hormonal stimuli), the PI [11] content of APM (23.6 μmol/mg protein) and TV (13.1 μmol/mg protein) would be involved in InsP3-mediated targeting of Ca to the specific fusion sites.

Recent studies by Fujimoto Toyoshi [17] reveal that a transmembrane protein structurally similar to the type-I IPR and the plasma membrane PM Ca-pump (Ca-ATPase) are concentrated in the caveolae. The HAF-stimulated H, K-ATPase activity under steady-state condition shows additional allosteric stimulation at very low level of Ca (<2 μM); beyond this the HAF-stimulated H, K-ATPase is drastically downregulated, coming to a halt at 4 μM. At this point the H-pump acts as a provisional Ca-pump to pump out excess Ca, thus resuming the H-pump activity (Fig. 4.4). Hence, higher Ca (2–4 μM) acts as a feedback control switch for turning off/on the HAF-operated H-pump as the top regulator [17, 18]. Type-I IPR in the ER is a Ca^{2+} channel that opens upon IP binding implicating that the caveolae associated IPR-like proteins are most likely plasma membrane Ca-channel regulating the intracellular Ca. In capillary endothelium the caveolae are closely related to the endoplasmic reticulum (ER) and it was suggested that the non-muscle cells storing Ca in ER should have similar relation [18].

In the case of parietal cells, the intracellular TV/BLM pool stores Ca, hence should have similar caveolae-connection mentioned above. So, the alteration in local Ca concentration should influence the nearby caveolae protein to be involved

in Ca-transport. In the case of parietal cell, Perez et al. [13] demonstrated (using the fluorescent signal of Fura-2) a Ca-transient at the secretary APM prior to the onset of acid secretion; the acid secretion occurred 3 s after carbachol stimulation. Simultaneously, a different group, Caroppo et al. [14] demonstrated using immunohistochemical staining the actual colocalization of H, K-ATPase, Ca-ATPase, and CaR in both the APM and TV membranes of parietal cell. It must be noted, however, that CaR was not detected in the BLM by these authors. Similar coexistence of PMCA and CaR was also revealed in the peptic cells, but not in any other cells like the mucus secreting and surface epithelial cells of gastric mucosa [14]. The parietal cells extrude Ca from cytosol through the APM into lumen, and take up Ca from the nutrient side coming through the BLM, hence is consistent with the absence of CaR in BLM just mentioned. Pancreatic acinar cells and salivary glands display similar initial increase in Ca at the lumen followed by Ca-wave spreading towards the BLM, consistent with immunochemical localization of PMCA on APM [19]. Thus, even though the BLM (analogous to APM) has provisional Ca-ATPase pump functioning as an altered form of the HAF-regulated Na-pump, there is no evidence of CaR involvement.

Thus, it is clear that the observed "Ca-transients" and CaR are intimately related. During the transfer of new proton-pump (from TV) on to APM, the newly incorporated pump will have Ca leftover (at fusion site) near the catalytic center that is pumped out at first by the provisional Ca stimulated Mg-ATPase (discussed earlier) for its subsequent operation as the HAF-regulated proton-pump. At this point, the CaR (facing the lumen) by way of its Ca-sensing devices [20–22] is likely to act as a sensor of cytosolic Ca and program itself as a regulator of the forthcoming Ca-transport events from the provisional Ca-pump across the bilayer. As soon as the CaR senses the unwanted level of Ca, the proton-pump turns into a provisional Ca-pump until the safe Ca level is reached. Similar role of CaR has been suggested in the voltage-gated channeling of Ca in the nerve terminal [5]. The detailed function of CaR as a Ca-sensor, a self-programmed timer, as well as a fine regulator of the Ca-channel function remains to be elucidated.

7 Emerging Picture of Ca-Signaling in Maintaining the Ionic Homeostasis

In view of the preceding information, and our data on the up- and down-regulation of the activator-regulated allosteric P2-ATPase pumps by μM Ca, the following unified picture is emerging:

- Calcium signaling is the absolute controller of homeostasis of our bodily cells equipped with allosteric P2-ATPase pump.
- The entire bodily network is operated by two different cytosolic regulatory proteins, namely the NaAF (170 kDa) and HAF (80 kDa) for the ubiquitous Na, K-ATPase and the distinctive gastric H, K-ATPase (proton-pump) respectively.

- Ca effects vary with local pH; the apical pump (operating at pH, 6.8) is shut off at 4 μM Ca while the basolateral (operating at pH > 8.0) needs five- to sixfold higher Ca for shutting off.

It may be noted that there are a lot of information in the literature dealing with the natures of the PMCA isoforms in Ca-signaling [23]. Based on current report, the tissue-specific isoforms of the plasma membrane Na-pump act as provisional Ca-pump (or PMCA) to pump out excess local Ca for maintaining homeostasis. So, the nature of the isoforms of the PMCA and Na-pump in any particular tissue should be identical. The current report will thus help in such tissue-specific identification of the related pumps as well as in identifying the area-specific isoforms of the P2-ATPase system in brain function [24].

8 Conclusions

The endogenous HAF is an allosteric regulator of the gastric H, K-ATPase system, which also seems to regulate its own intracellular level by regulating gene expression [25]. This chapter reveals that the HAF-regulated H, K-ATPase system is, in turn, allosterically regulated by the cytosolic free Ca in a pH-dependent manner. It will be important to know if Ca has any feedback influence on the genetic self-regulation scheme of the HAF that is so vital in allosteric ion transport by the gastric H, K-ATPase system.

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