FEBS Letters 584 (2010) 2102–2111

journal homepage: www.FEBSLetters.org

Review

Chloride channels of intracellular membranes

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ARTICLE INFO

Article history:
Received 21 December 2009
Revised 13 January 2010
Accepted 19 January 2010
Available online 26 January 2010

Edited by Adam Szewczyk

Keywords:
Chloride channel
ClC
CLIC
Bestrophin
Golgi pH regulator

ABSTRACT

Proteins implicated as intracellular chloride channels include the intracellular ClC proteins, the bestrophins, the cystic fibrosis transmembrane conductance regulator, the CLICs, and the recently described Golgi pH regulator. This paper examines current hypotheses regarding roles of intracellular chloride channels and reviews the evidence supporting a role in intracellular chloride transport for each of these proteins.

1. Introduction

The study of chloride channels of intracellular membranes has seen enormous advances over the past two decades and exciting recent developments have sparked renewed interest in this field. The discovery of important roles for intracellular chloride channels in human disease processes as diverse as retinal macular dystrophy, osteopetrosis, renal proximal tubule dysfunction, and angiogenesis have highlighted the importance of these molecules in critical cellular activities. Startling discoveries regarding the intracellular ClC family of proteins have forced a re-examination of some of the fundamental assumptions regarding acidification of intracellular organelles. Newly discovered channels have attracted intense interest and the importance of long-recognized proteins has been questioned.

Investigations of intracellular channels present unique technical challenges. Perhaps most importantly, channels expressed exclusively on intracellular membranes are largely inaccessible to the direct application of the powerful patch-clamp techniques that led to rapid characterization of plasma membrane ion channels. As an alternative, intracellular channels can be studied in isolated vesicle fractions, but membrane fractionation techniques are always imperfect, with unavoidable contamination of membranes prepared from one organelle with those from other compartments. While low level contamination may not be critical to typical biochemical studies, contamination can be a fatal confounder in single molecule assays such as single channel recordings. These and other technical obstacles have impeded progress. Nonetheless, anion conductances have been demonstrated in numerous intracellular compartments and a host of discreet chloride channel activities have been described [1–3].

Perhaps counter-intuitively, the regulation of concentration or amount of chloride itself within compartments has not been seen as the major role of these intracellular chloride channels. Instead, the key role of chloride permeability has been thought to be to function as a short-circuiting conductance to allow transport by electrogenic cation transport mechanisms. For example, acidification of intracellular organelles by the electrogenic proton ATPase is recognized as a process that requires a short-circuiting conductance to allow transmembrane cation transport [4,5]. Other processes which may require a chloride short-circuiting conductance across intracellular membranes include calcium transport across the sarcoplasmic reticulum (SR) [3,6] and potassium influx into secretory vesicles [7].

While earlier investigations established the presence and possible roles of chloride conductances in intracellular organelles, more recent studies have tended to focus on identification of the molecular components responsible for these activities. Several proteins have now independently been implicated in intracellular chloride conductances, including ClC family members, the cystic fibrosis

Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; GPHR, Golgi pH regulator; SR, sarcoplasmic reticulum; ER, endoplasmic reticulum

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0014-5793/$36.00
doi:10.1016/j.febslet.2010.01.037

transmembrane conductance regulator (CFTR), bestrophins, the CLIC family, and the recently described Golgi pH regulator (GPHR). In this review, we will first briefly consider some physiological functions for chloride channels of intracellular organelles and then examine data supporting the roles of each of the protein families listed above. Intracellular roles for ClCs, CFTR, and the bestrophins have been the subjects of recent reviews and will be summarized only briefly. Evidence for a role of CLICs as intracellular chloride channels will be examined in more detail. This review will not address mitochondrial porins or VDAC.

2. Roles for intracellular chloride channels

2.1. Acidification of intracellular compartments

Most intracellular compartments maintain a steady-state pH somewhat more acidic than the cytoplasm, varying from about 6.5 in early endosomes and the Golgi apparatus to as low as 4.5 in mature lysosomes [8]. This low pH is implicated in numerous intraluminal events including dissociation of ligand/receptor complexes along the endosomal/recycling pathway, activation of hydrolytic enzymes in lysosomes, appropriate post-translational modification of secreted proteins in the Golgi and trans-Golgi network, and loading of neurotransmitter vesicles [5,9,10]. In addition to these intraluminal actions, acidification also appears to be essential for membrane traffic itself. Blocking acidification results in cessation of membrane traffic, perhaps due to an essential role for low luminal pH in membrane fusion events, for recruiting components of the fusion apparatus to the vesicle, or in modulation of transmembrane components of the fusion apparatus [10,11].

Acidification of intracellular compartments primarily occurs through actions of the vacuolar proton ATPase acting in parallel with a chloride conductance [12]. The pump is electrogenic, moving a hydrogen ion across the membrane using the free energy released by hydrolysis of ATP. It is clear from consideration of the free energy available to the pump and estimates of luminal buffering power and membrane capacitance of typical vesicles that actions of the pump alone in the absence of other leak mechanisms would lead to generation of an electrical potential but no significant proton gradient [13]. The chloride conductance short-circuits the electrical potential and allows the pump to generate a pH gradient. Since the chloride conductance is essential to allow acid transport, several authors have suggested that regulation of the chloride conductance could be one means of regulating compartmental pH [14–16], but whether the chloride conductance contributes to active regulation of pH of intracellular compartments remains uncertain. Several independent studies have concluded that at least for phagosomes, lysosomes, and the Golgi, anion conductance is not limiting for acidification and that steady-state pH is primarily regulated by proton pump and leak rates [17–21]. Although the role of the proton ATPase in vesicular acidification is firmly established, recent evidence that ClC-5 functions as a chloride–proton exchanger rather than a channel suggests that non-H-ATPase dependent acidification mechanisms also may contribute under certain circumstances [22,23].

2.2. Release of Ca from endoplasmic and sarcoplasmic reticuli

Cycles of regulated release of calcium from SR via calcium-release channels followed by reuptake of calcium by a calcium ATPase are key steps in muscle contraction [6]. Similar processes occur across endoplasmic reticulum (ER) membranes in non-muscle cells. Both calcium release and reuptake are electrogenic processes that require counterion movement to allow mass transfer of calcium. Chloride channels are present in the SR but their precise role is uncertain [3,6,24]. The major calcium-release channel of SR is the ryanodine receptor. Calcium release via ryanodine receptor in various experimental models does not require the presence of chloride or other permeable anions [24]. Hence the counterion movement is thought to be carried by cations, primarily potassium, although a contribution by chloride in vivo has not been conclusively disproven. A recent theoretical analysis suggests that the cation counterion current may be carried by the ryanodine receptor itself by virtue of its low ion selectivity [24]. Active calcium uptake into the ER/SR by the calcium ATPase is also an electrogenic process that requires counterion movement that may be supplied by chloride channels [25].

2.3. Exocytosis of secretory vesicles

The roles of ion channels in exoctyosis have been reviewed in detail [7]. The mechanistic diversity of exocytosis among various models systems of interest defy easy generalization. In brief, chloride channels are known to be present in essentially all secretory vesicles that have been studied. Many of these vesicles acidify at least transiently along the exocytic pathway and one role of chloride conductances in these vesicles is to support acidification. This acidification may have particular exocytic-specific roles in individual cell types. For instance, acidification is important for loading of certain neurotransmitter vesicles [9] and for processing of insulin in secretory vesicles of pancreatic β cells [26]. Furthermore, acidification supported by a chloride conductance appears to play a role in exoctyosis itself in pancreatic β cells [7]. In contrast, mature zymogen granules of the exocrine pancreas are not acidic and exocytosis does not require acidification [27]. Zymogen granule membranes contain potassium and chloride conductances which are activated during exocytosis and inhibitors of these channels inhibit exocytosis. Exactly how these channels support exocytosis remains uncertain [7].

2.4. Chloride channels of mitochondria

Mitochondria contain anion channel activities in both the inner and outer membranes [28]. The primary conductance of the outer membrane, the Voltage Dependent Anion Channel (VDAC), is more properly thought of as a porin rather than a typical ion-selective channel and is responsible for movement of anions, cations, and non-electrolyte metabolites across the outer membrane [29]. Ion permeability of the inner membrane is tightly regulated and needs to be very low under ATP-synthesizing conditions to allow the pH and electrical gradient generated by electron transfer chain to drive ATP synthesis by the mitochondrial ATPase. Nonetheless, the inner membrane does contain a chloride channel activity known as the inner membrane anion channel (IMAC) and single channel studies have identified discreet channel activities which may account for this conductance [30,31] although the proteins responsible are unknown. The IMAC activity is thought to contribute, in parallel with a potassium conductance, to mitochondrial volume regulation [28] and to oxidative stress-related inner membrane depolarization [32]. A second anion conductance of the inner membrane is associated with the uncoupling protein UCP. UCP functions as a proton and chloride leak mechanism that uncouples the electron transport chain from ATP synthesis and leads to heat generation from mitochondria in brown fat [28]. Most recently, an inner membrane high conductance anion selective channel has been reported which is postulated to be related to the Permeability Transition Pore [33] although its role in development of this non-selective permeabilization is uncertain.