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ROLE OF PROTONS IN CALCIUM SIGNALING

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ABSTRACT

36 years after the publication of the important article by Busa and Nuccitelli on the variability of intracellular pH (pH_i) and the interdependence of pH_i and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), little research has been carried out on pH_i and calcium signaling. Moreover, the results appear to be contradictory.
Some authors claim that the increase in $[Ca^{2+}]_i$ is due to a reduction in pH_i, others that it is caused by an increase in pH_i.
The reasons for these conflicting results have not yet been discussed and clarified in an exhaustive manner.
Variations in pH_i have a significant impact on the increase in $[Ca^{2+}]_i$ and hence on some of the basic biochemical mechanisms of calcium signaling. This paper focuses on the possible triggering role of protons, highlighting the mechanisms potentially involved and the open issues that could be clarified by research.

Keywords
Ca-signaling; IP_3; RyR; intracellular-pH; $[Ca^{2+}]_i$; proton.

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1. INTRODUCTION

The value of intracellular pH (pH_i) is the measure of the concentration of protons in the cell ($[H^+]_i$) and indicates the acid or alkaline nature of the cytoplasm. We are aware that this notation is generic, because it does not distinguish between the concentrations $[H^+]$ of the various cellular compartments. Referring to cytosol and using the notation $[H^+]_c$ would be more precise, but we prefer to keep using the notation $[H^+]_i$ in consideration of previous publications [Busa and Nuccitelli 1984]. Normally, the pH_i value is about 7.2, i.e. slightly alkaline. On the basis of experimental data, until the early 1980s, it was thought that the pH_i value was essentially stable. In 1984, an invited opinion was published which dismantled this hypothesis and shifted the point of view on the question [Busa and Nuccitelli 1984].

Comparing a huge quantity of data from the publications then available, the authors showed that, on the contrary, pH_i varies significantly. Their study includes a table [Busa and Nuccitelli 1984, Table 1] with numerous events in the life of a cell associated with variations in pH_i, including developmental transitions and stimulus-response coupling, in which calcium signaling is a fundamental step. After analyzing the experimental data, Busa and Nuccitelli declared that the intracellular concentration of H^+ ($[H^+]_i$) is related to the intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$). Their results indicate correlations that are not always in the same direction, sometimes with trends that are unexpected or even the reverse of expectation. Indeed, they observe: *"mounting evidence suggests that pH_i and Ca^{2+} changes can be interdependent, both in their mechanisms and their effects, although the significance of such interactions is still largely unclear"*.

Clearly, this is an important finding, both in general terms relating to cellular processes and specifically in relation to calcium signaling. And yet, after the publication of this paper, few articles appeared on the interdependence of concentrations of pH_i and $Ca^{2+}{}_i$ and the results were partly contradictory [Austin and Wray 2000] for reasons that are still unclear and deserve investigation for the purposes of clarification.

For some time, studies on the role of protons in cellular processes have taken into consideration the relationship between pH and calcium and its effects, in particular in relation to cellular channels and organelles. Protons modulate the action of various types of channel, including voltage-gated proton channels [DeCoursey 2018] which extrude protons, the Orai/STIM and transient receptor potential (TRP) channels [Huang 2010, Yu 2018, Vangeel 2019], which foster Ca^{2+} influx via store-operated calcium entry (SOCE), and two pore channels (TPC) [Kintzer 2018, Galione 2019]. Sarco-endoplasmic reticulum Ca^{2+}-ATPase pump (SERCA) and the plasma membrane Ca^{2+}-ATPase pump (PMCA) remove excess Ca^{2+} from the cytosol. The former conveys it to endo/sarcoplasmic reticular stores [Primeau 2018], the latter takes it outside the cell, exchanging it with a proton [Brini 2011, Hegedűs 2020]. The action of the PMCA would acidify the cytosol were it not for the counterbalancing action of the Na^+/H^+ exchangers, which expel the proton in exchange for a Na^+ ion [Brini 2011, Xu 2015, Yang 2019]. The protons are also removed from the cytosol by various kinds of Vacuolar H^+-ATPase pump (V-ATPase), which can transport them to the organelles or outside the cell, exchanging them with various cations, including Ca^{2+} [Fogarc 2007, Colina-Tenorio 2018, Faris 2019]. The ATP which provides the energy required for the functioning of the PMCA and SERCA pumps is produced in the mitochondria by virtue of the protons, which – according to chemiosmotic theory – enable the entry of Ca^{2+} into the mitochondria [Pallafacchina 2018, Wilson 2019]. The proton gradient is the basis of the movement of the molecular rotary motors both in the mitochondria and the V-ATPases [Stewart 2015, Colina-Tenorio 2018].

Therefore, protons clearly play a fundamental role in metabolic processes, in particular in the organelles in which Ca^{2+} is accumulated. The cellular homeostasis of the calcium requires balance and continuous interchange between the organelles and the cytoplasm and between the latter and the extracellular space. This is possible only through the concerted action of the channels, pumps, and exchangers as described.

In relation to calcium signaling, it is believed that protons have a mainly regulatory role [Worley

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1987, Donoso 1996, Felle 2001, Poburko 2011, Raffaello 2016, Yu 2018, Yang 2019]. To our knowledge, no study explicitly attributes a triggering role to protons or makes an in-depth biochemical investigation of the interdependence mechanism between the H^+ and Ca^{2+} ions. Nonetheless, significant progress has been made over the past 40 years or so: several fundamental second messengers and pathways for calcium signaling have been identified [Pozzan 1994, Clapham 2007, Berridge 2012, Balla 2013, Carafoli 2016, Galione 2019], and the understanding of specific receptors [Rossi 2018, Prole 2019] and channels [Kozak and Putney 2017, Galione 2019] has improved. Experimental methods, particularly calcium imaging techniques, have been developed [Costa 2018, Liu 2018, Depaoli 2019, Siciliano 2019, Bischof 2019, Greotti 2020]. It has been shown that stimulus induces characteristic transient calcium blips, puffs (more generically known as calcium sparks or spikes) [Rose 2006], which in turn, through repetition, can give rise to calcium waves and oscillations [Dupont 2011, Gaspers 2014]. Despite this, some interactions between the various molecular players remain obscure [Raffaello 2016, Ribeiro 2018]. For example, we do not fully understand the biochemical mechanism by which second messengers such as inositol 1,4,5-trisphosphate (IP_3) mobilize the bound calcium of cellular stores, hence the resulting increase in $[Ca^{2+}]_i$ concentration and the appearance of calcium spikes. The origin of an important event such as "Ca^{2+} Induced Ca^{2+} Release" (CICR) is also unknown.
To assess and understand the role of the molecular players involved, a better understanding of the biochemical mechanisms involved in their interactions is required. Specifically, further experimental research is needed to properly assess the importance of the variation in the intracellular concentration of protons $[H^+]_i$, measured as a change in pH_i, in relation to the variation in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and the consequent activation of some enzymes.

2. ABOUT OUR RESEARCH

This work sets out to illustrate the biochemical mechanisms underlying H^+/Ca^{2+} interconnections in the cell, focusing on the effects of variations in the concentration of protons on the change in $[Ca^{2+}]_i$ concentration. Our goal is to draw attention to some neglected points rather than to provide a complete review. The chemical properties of the H^+ and Ca^{2+} ions, their interdependence and the effects at the cellular level suggest that protons have an active role in terms of stimulation, not only of control and regulation, and are therefore fundamental in calcium signal transmission. This hypothesis shows the mechanisms of calcium signaling in a new, broader perspective, and leads to a better understanding of numerous aspects and processes hitherto unclear.
Scientific literature available on-line from 1970 to February 2020 was drawn upon to analyze data and critically evaluate the fundamental concepts involved in the topic under study.

3. H^+/Ca^{2+} INTERDEPENDENCE

3.1. H^+/Ca^{2+} interdependence in aqueous solution

First, it is worth remembering that in aqueous solution, H^+/Ca^{2+} interdependence is a direct consequence of the chemical properties of two elements, hydrogen and calcium. In its protonated form, hydrogen (H^+) with anions give rise to acid molecules which are generally soluble in water. When Ca^{2+} binds with the anions, it forms salts, but unlike other positive ions such as K^+, Na^+ and NH_4^+ which produce soluble salts, Ca^{2+} can give rise to insoluble salts [MacConaill 1985, Roth-Bassel 1992, Periodic Table Solubility 2019]. For example, it is possible to verify in the lab that a solution of a sufficient quantity of Ca^{2+} in deionized water at alkaline pH is murky due to the formation of miniscule solid particles of insoluble salts suspended in the solution. This occurs because the Ca^{2+} binding to the OH^- of the water and to the dissolved CO_2forms insoluble calcium hydroxide and calcium carbonate respectively. All the more, a solution containing ions

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physiologically present in the cell cytosol is murky because the Ca^{2+} binding to the inorganic (hydroxide, carbonate, phosphate, sulphate) and organic (oxalate, citrate, peptidate, proteinate) anions of the cytosol tends to form corresponding insoluble salts and complex compounds [Hincke 1978, MacConaill 1985, Iida 1986, Dubois 1990, Donoso 1996, Goss 2010]. The quantity of insoluble compounds formed which tends to precipitate depends on a number of factors: solubility product, ionic strength, ionic radius, coordination number, temperature and, above all, pH. The solubility of calcium salts in water is pH-dependent because the protons compete with the calcium for the same binding sites and can easily replace it. This replacement increases calcium-free Ca^{2+} in the solution reducing its murkiness. Therefore, adding a drop of acid with a concentration 10-20 times that of the total calcium present, it is possible to check quite easily for a reduction in murkiness and simultaneous increase in Ca^{2+} ions in the solution. This reaction, which can be seen by the naked eye, is transitory and localized, because it involves a volume that is just a little larger than the drop of acid added, and can therefore be described as a calcium spike on a macroscopic scale. To conclude, the murkiness increases when the pH is moved towards alkaline (=basic) values and decreases when the pH moves towards acid values. These localized and transitory variations in murkiness are evident even for small variations in pH, especially when buffer systems are present as in the physiological-like solution. On the other hand soluble Ca^{2+}-bound proteins do not produce murkiness; in these cases protons reduce the binding affinity of ligands and promote Ca^{2+} release. In conclusion, an increase in alkalinity (increase in pH) fosters the formation and storage of bound calcium compounds and hence a decrease in the concentration of free calcium ions [Ca^{2+}], whilst an increase in acidity (decrease in pH) fosters the solubilization/breaking of these compounds and hence an increase in Ca^{2+} concentration. Therefore, there is a direct chemical interdependence between H^+ and Ca^{2+} concentrations so, in a closed system, when one increases so does the other and vice versa. This direct and proportional interdependence between [H^+] and [Ca^{2+}] has been shown experimentally in skeletal muscles [Donoso 1996] and in blood [McCudden 2013]. The relationship is shown by McCudden in Fig. 1, clearly illustrating the concept.

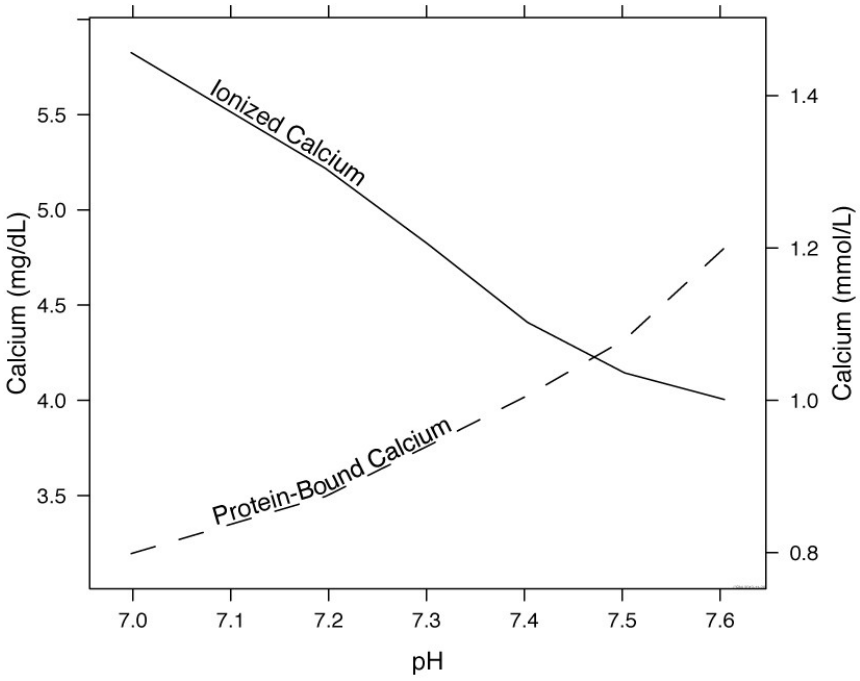

FIG. 1: The effect of pH on protein-bound and free calcium in blood.
(By Christopher R. McCudden and reproduced here with kind permission of Radiometer).
"As pH decreases, H^+ displaces Ca^{2+} from binding sites and the amount of free Ca^{2+} increases. Conversely, as the blood pH increases, albumin and the globulins become more negatively charged and bind more calcium, causing the amount of free Ca^{2+} circulating to decrease."

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It is important to note that in aqueous solution, the above pH-dependent reactions, i.e. calcium storage and calcium release may occur rapidly and spontaneously in the absence of biological structures, because they are the consequence of fundamental chemical laws. For example, it is possible to obtain the appearance of a calcium spike, on a macroscopic or microscopic scale, simply by localized acidification. To trigger the reactions of calcium storage and calcium release in aqueous solution, a pH change is sufficient, caused by any event that reduces or increases protons. A clear understanding of what can and cannot happen in aqueous solution due to the chemical interdependence between concentrations of H^+ and Ca^{2+} ions enables a more complete and coherent assessment of the data obtained experimentally due to variations in pH_i in the cell.

3.2. H^+/Ca^{2+} interdependence in the cell

The relationship between H^+ and Ca^{2+} integrated in the biochemical process inside the cell should be considered as part of the overall metabolic system of the cytoplasm.
It could be expected that, similarly to what occurs in an aqueous solution, the acidification of cellular cytosol always produces the depletion of calcium stores and consequently increases $[Ca^{2+}]_i$ concentration and that, on the other hand, alkalinization always produces the formation and storage of bound calcium and therefore decreases $[Ca^{2+}]_i$ concentration. If this were the case, all the results of experimental studies on the effects of variations in pH_i (decrease or increase of protons) on $[Ca^{2+}]_i$ concentration in the cell would be consistent. But experiments have produced contrasting results. Some show that the increase in $[Ca^{2+}]_i$ is produced by acidification, in line with expectations given the chemical properties described above [Orchard 1987, Kim 1988, Cairns 1993, Daugirdas 1995, Donoso 1996, Nishiguchi 1997, Nagaoka 1997, González 1998, Balnave 2000, Chen 2001, Thomas 2002, Marin 2010, Krizaj 2011, Paillamanque 2016, Hu 2017, Behera 2018, Berra-Romani 2019]. Other results, on the contrary, have shown that the increase in $[Ca^{2+}]_i$ is produced by alkalinization [Kiang 1991, Guse 1994, Nitschke 1996, Lindeman 1998, Minelli 2000, Alfonso 2000, Li 2012].
The situation in the cell is highly complex due to the presence of numerous biological structures (organelles, channels, pumps, receptors) which control and regulate ionic concentrations, in particular $[Ca^{2+}]_i$ concentration, modifying them according to physiological needs. Physiological processes can influence H^+/Ca^{2+} interdependence fostering or resisting it, pumping H^+ and/or Ca^{2+} outside the cell or in the organelles, and producing/consuming protons [Wacquier 2016, Raffaello 2016, Szymanski 2017, Faris 2019].
Therefore, in relation to the contrasting experimental results referred to above, it could be thought that a biochemical process, activated out of need by the cell, and hence extraordinarily, prevails over the ordinary chemical process producing a different result. This process should be identified and the reasons for the need explained. Only a few studies have provided an explanation by attributing the cause of the increase in $[Ca^{2+}]_i$ produced by alkalinization to inhibition of the SERCA pump [Li 2012] or to a calcium influx [Lindeman 1998, Alfonso 2000, Minelli 2000].
To clarify this, some critical factors need to be taken into consideration, above all, response times. It is likely that studies of events with a medium/long-term response, such as those relating to situations of acidosis or alkalosis of the organism, involve mechanisms that are partly different from those with short-term responses. A medium/long-term response is more susceptible to outside influences and is more difficult to interpret, whilst a rapid response is usually sharper and clearer. The time between the stimulus and appearance/disappearance of the calcium spike is a matter of seconds. The times are shorter in animal cells than in plant cells and excitable cells compared to non-excitable cells. In Xenopus Oocytes, which are excitable cells, the mean rise time of puffs is 19 ms and they are often preceded by trigger events with average durations of ~12 ms [Rose 2006]. The time of neutralization of an acid with a base is a matter of µs [Donten 2012] and the

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dissociation of a weak acid, i.e. the time for the release of an H^+ ion, is even quicker [Kanzaki 2014].
To identify and explain the mechanism underlying calcium signaling, it is therefore necessary to understand what happens in an extremely short time. At the experimental level, resources are required, specifically sensitive reagents and instruments able to respond to this speed of reaction.
The reagents are the second critical factor, because they can produce interference, producing variations in the process. In studies of H^+/Ca^{2+} interdependence, variations in pH_i should be induced as far as possible physiologically, not by the introduction from outside of artifacts or acid compounds with buffering, chelating and/or permeabilizing effects or indicators that could modify the original pH_i and $[Ca^{2+}]_i$ or the level of calcium bonded in cellular stores.
The third critical factor comprises the instruments and equipment used. Over the years, many different instruments and techniques have been used, often not dedicated and/or insufficiently sensitive. To overcome these limits, multiple complementary techniques and pharmacological manipulation are required [Bird 2018]. Chemical indicators, including Fluo-4 acetoxymethyl (AM), Rhod-2 AM, Fura-2 AM and the Ca^{2+} chelator BAPTA, commonly used over the past 25 years, are nowadays considered by some authors to be excessively invasive [Smith 2018]. But even modern genetically-encoded Ca^{2+} indicators have their problems [Bootman 2018, Yang 2018]. Given the different experimental methods, it is not surprising that the results are conflicting and not comparable.
The fundamental concepts acquired and currently shared by the scientific community in relation to calcium signaling are increasingly based on the key role of Ca^{2+} in the cell metabolism. For good ordinary functioning, the cell maintains a $[Ca^{2+}]_i$ concentration of 0.1 µM [Bootman 2019] through the action of PMCA and SERCA pumps, which expel excess Ca^{2+} from the cytosol. The increase of $[Ca^{2+}]_i$ from 0.1 to 1 µM and beyond, essential for the transmission of the signal, but excessive and potentially toxic for the cell, is an extraordinary and temporary event. This event is triggered by an external stimulus which, through the activation of a specific receptor, induces the release of a second messenger, in turn inducing calcium store depletion, the formation of calcium spikes and/or waves and, finally, calcium influx [Bootman 2019]. How the second messenger causes the release of Ca^{2+} from calcium stores, the formation of calcium spikes and increase in $[Ca^{2+}]_i$ concentration is unknown.
However, it may be that via the chemical properties of the H^+ and Ca^{2+} ions described in the section "3.1. H^+/Ca^{2+} interdependence in aqueous solution", the protons freed by the second messenger [Molinari 2015] foster the release of calcium, replacing it in cellular stores. The following paragraph describes this hypothesis in detail, focusing on the PLC/IP_3 pathway.

4. THE DUAL MOLECULAR MECHANISM FOR IP_3 TRIGGERING

The PLC/IP_3 pathway is triggered by an external stimulus, via agonist-receptor interaction on the plasma membrane (PM), activating phospholipase C (PLC).
Generally, the receptor is in one of the following types: G-Protein-Coupled Receptors (GPCR), Tyrosine Kinase Receptors (RTK), or Tyrosine Kinase Non-Receptors (NRTK). In most cases, the PLCs belong to isoform PLC-β, PLC-γ, and PLC-ε [Cocco 2015, Nakamura 2017].
All the phospholipases are heavily acidifying enzymes, because they hydrolytically split the lipid esters, producing an alcohol and an acid [Molinari 2019].
In particular, PLCs hydrolyze the phosphatidyl-inositol bisphosphate (PIP_2) into 1,2-diacylglycerol (DAG) plus IP_3 [Michell 1981, Streb 1983]. The DAG is the alcohol and the IP_3 the acid. The newly generated IP_3 is protonated and has the following formula: $C_6H_{10}O_{15}P_3^{5-}$.

(1) $PIP_2 + H_2O \rightleftharpoons DAG + IP_3$
native IP_3 ($C_6H_{10}O_{15}P_3^{5-}$)

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The native IP_3 must almost completely dissociate to bring itself into balance with the pH=7.2 of the cytosol and hence quickly releases a proton:

(2) $C_6H_{10}O_{15}P_3^{5-}$ (native IP_3) $\rightleftharpoons$ $C_6H_9O_{15}P_3^{6-}$ (dissociated IP_3) + H^+

The protons produced by the dissociation of the IP_3, following hydrolysis of PIP_2, can reduce the affinity of Ca^{2+} for binders, such as inorganic anions or proteins, which immobilize it in the cytosol, and can foster the release of Ca^{2+} triggering rapid calcium spikes. It has been shown experimentally that the stimulus causes in the cytosol an increased concentration of protons and, in parallel, of Ca^{2+} [Kim 1988, Daugirdas 1995, Paillamanque 2016, Behera 2018, Berra-Romani 2019]. The cellular control mechanisms and first of all the cytosolic buffering capacity respond similarly quickly, ending the spikes and returning the situation to a stationary state.
The rapid increase and subsequent decrease in $[Ca^{2+}]_i$ concentration, characteristic of transient calcium spikes, can be attributed to the chemical properties of the H^+ and Ca^{2+} ions and their interdependence, as shown by Busa and Nuccitelli.
Subsequently the IP_3 activates its receptor (IP_3R), which for most cells is located on the membrane of the endoplasmic reticulum (ER) [Thillaiappan 2017]. There are three types of IP_3R with quite similar characteristics [Vanderheyden 2009]. The activation of IP_3Rs by IP_3 induces the rapid release of Ca^{2+} from the calcium stores of the ER [Berridge 2012, Rossi 2018, Prole 2019]. For the first time in 2017 the correlation of subsequent events "acid stimulus - PLC/IP_3 transmission - Ca^{2+} release from ER" was experimentally proved [Hu 2017].
The ability of calcium to stimulate the release of further calcium was observed for the first time in the 1970s, before the discovery of IP_3. The phenomenon is known as Ca^{2+} Induced Ca^{2+} Release (CICR) and is discussed in detail below. The molecular mechanism of CICR is unknown as is the gating mechanism of IP_3R channels, activated by IP_3, enabling the release of calcium from the endo/sarcoplasmic reticulum [Chandran 2019, Prole 2019, Hamada 2020].
IP_3R terminates the action of IP_3 through its phosphorylation by inositol 1,4,5-trisphosphate 3-kinase to inositol 1,3,4,5-tetrakisphosphate or its degradation by inositol polyphosphate 5-phosphatase (5PTase) to inositol 1,4-bisphosphate [Majerus 1992, Sims 1998, Berdy 2001, Pattni 2004, Balla 2013, Hsu 2015]. The latter pathway could be prevalent, since in bovine tracheal smooth muscle the 5PTase pathway was shown to be the dominant route in metabolism of IP_3 "*at all time intervals measured, especially at early times (0–300 sec), where it accounted for more than 85%*" of IP_3 metabolism [Lynch 1997].
5PTases are found in plants, animals and fungi; they possess phylogenetic relationships, but some different substrate specificities. In plants they are classified into five families [Jia 2019], while mammalians 5PTases have been classified into four groups [Majerus 1999]. Dysfunctions of 5PTases are at the origin of various diseases in humans [Dyson 2012, Rudge 2016, Ramos 2019].
Studies on the action of 5PTases on IP_3 and Ca^{2+} in animals have produced results consistent with the involvement of 5PTases in Ca^{2+} release: when 5PTase is overexpressed, IP3 decreases and Ca^{2+} oscillations do not occur, on the contrary its underexpression leads to increased cellular levels of IP_3 and Ca^{2+} [Bui 2002, Mitchell 2002, Johenning 2004, Liu 2020]. At least five human 5PTases capable of degrading IP_3 are known: the Inositol polyphosphate-5-phosphatase A, belonging to group I, is located on the inner wall of the PM and/or in the cytosol and is active only on soluble IP_3 and inositol 1,3,4,5-tetrakisphosphate [Laxminarayan 1993, Mitchell 2002]; the other four 5PTases, namely Type II inositol 1,4,5-trisphosphate 5-phosphatase [Jefferson 1995, Speed 1995, Schmid 2004], its homolog Inositol polyphosphate 5-phosphatase OCRL [Zhang 1995, Schmid 2004, Metha 2014], Phosphatidylinositol 4,5-bisphosphate 5-phosphatase A [Mokizuki 1999], and Synaptojanin-1 [McPherson 1996, Johenning 2004] belong to group II and have a broader specificity since, in addition to IP_3, they can also remove the 5-phosphate from phosphatidylinositol 4,5-bisphosphate

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and inositol 1,3,4,5-tetrakisphosphate from soluble as well as insoluble substrates. These five human 5PTases are encoded by the INPP5A, INPP5B, OCRL, INPP5J and SYNJ1 genes respectively. None of them has been localized to ER or SR until now. A broad-specificity endoplasmic 5PTase, named SKIP or INPP5K, which included IP_3 among its possible substrates, was discovered in 2000 [Ijuin 2000]. Unfortunately, the IP_3 specificity of SKIP has not been confirmed by other authors [Gurung 2003, Schmid 2004]. So logically one wonders what the role of this enzyme is [Dong 2018]. Schmid et al. have provided a very interesting comparison of the activity and substrate specificity of some 5PTases, belonging to group II [Schmid 2004 Fig.1]. A similar comparison would be useful, with IP_3 as substrate, also extended to the group I PTase.
Based on the above data, it remains difficult for us to understand what is/are the main 5PTase(s) responsible of IP_3 degradation to inositol 1,4-bisphosphate mediated by IP_3R.
However, it is important to note that, like PLCs, phosphatases and hence also 5PTases, are strong acidifiers, because they hydrolyze phosphoric esters such as IP_3 producing an alcohol – the inositol 1,4-bisphosphate - and inorganic acid phosphate. The reaction is as follows:

(3) $C_6H_9O_{15}P_3^{6-}$ (dissociated inositol 1,4,5-trisphosphate (IP_3)) + H_2O ⇌ $C_6H_9O_{12}P_2^{5-}$ (inositol 1,4-bisphosphate) + $H_2PO_4^-$ (acid phosphate)

As soon as it is produced, the acid phosphate must dissociate almost completely to bring itself into balance with the pH of the cytosol and releases a proton, similarly to IP_3 as described above.

(4) $H_2PO_4^- \rightleftharpoons HPO_4^{2-} + H^+$

It is known that protons are important allosteric modulators and can produce changes in the conformation of proteins with considerable structural modifications [Onufriev 2013]. The multidentate Ca^{2+} binding promotes folding of the protein structure, while protonation of amino acid residues through Ca^{2+} replacement promotes electrostatic repulsion and consequent unfolding of the structure [Berggård 2000]. Functional consequences can be seen [Fan 2017]. The IP_3R in its structures and functions evokes other ligand-gated ion channels, as ionotropic glutamate receptors, some of which are subject to allosteric modulation by protons [Zhang 2018]. Interestingly, hystidine residues are involved in the allosteric modulation by protons and divalent ions of the gating of P2X7, a tetrameric, ligand-gated, purinergic channel [Acuña-Castillo 2007].
The study of the gating mechanisms of IP_3R is difficult, because the distance between the IP_3 binding site, facing the cytosol, and the Ca^{2+} conducting pore, facing the ER lumen, is relatively long. The mechanism is not yet completely understood and the structures of opened channels were never obtained. However, we know that the IP_3-dependent mechanism of IP_3R gating needs a certain level of Ca^{2+}: the activity is boosted by slight increases in $[Ca^{2+}]_i$ and is inhibited by significant increases [Bezprozvanny 1991, Foskett 2007, Prole 2019].
The structures currently available of IP_3R determined by electron cryomicroscopy and X-ray crystallography prove that: IP_3R is tetrameric and requires four IP_3 molecules in addition to Ca^{2+} to open the channel [Hamada 2020]; the gating is due to reversible conformational changes of the structure; the tetramer faces the cytosolic side [Hamada 2020] and so it is accessible to both cytosolic phosphatases and cytosolic protons. Moreover, the binding of IP_3 breaks the hydrogen bonds between the amino acid residues of IP_3-binding core and causes a rearrangement that leads to the allosteric opening of the Ca^{2+} conducting pore [Chandran 2019]. Probably, the 5-phosphate group of IP_3 is also involved, because flipping of hydrogen bonding between the 1-phosphate and 5-phosphate groups of IP_3 has been observed [Chandran 2019].
Since the degradation of IP_3 by 5PTase concerns the 5-phosphate group, it is possible to think that the flipping reflects the enzymatic action. Furthermore, the protons produced by IP_3 as a result of its degradation could modulate the amount of bound Ca^{2+} in IP_3R and induce the conformation changes

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necessary to open the channel. Consequently, for the first time, we put forward the hypothesis that 5PTases, by degrading IP_3, enable the action of IP_3 when the hydrolysis ends, freeing protons, opening the IP_3R channel and releasing Ca^{2+} from ER stores.
In summary, the action of IP_3 could take place in the cell, via the release of protons, at two different moments and in different points:

1) at the moment in which, via the PLCs, IP_3 frees a limited but significant quantity of H^+ and Ca^{2+} on the inner wall of the PM [reactions (1) and (2)];
2) at the moment in which IP_3 is degraded by the 5PTases on the outer side of the ER membrane or close by, with the consequent release, via the IP_3R, of a larger quantity of H^+ and Ca^{2+} [reactions (3) and (4)].

The two events are not mutually exclusive, and are likely inter-related as well as related to the control mechanisms that, via STIM and ORAI, activate the Ca^{2+} influx at the next phase of calcium signaling.
Since in some cells, such as oocytes, the 5PTases are mainly cytosolic enzymes and IP_3Rs are present in various ways throughout the cytoplasm [Yoon 2019], it is possible that in some cases the activation of IP_3R occurs in places other than that specified in point 2 above and involves the entire cytoplasmic space.

The hypothesis summarized above in points 1 and 2 is apparently simple, but has numerous implications. It is based on three fundamental and experimentally consolidated concepts:

- The activation of hydrolytic enzymes, such as phospholipases and phosphatases, produces acids.
- Via their protons, the acids foster the solubilization and release of bound calcium, freeing Ca^{2+}.
- Due to the high solubility and small size of the H^+ ion, protons are much more mobile than Ca^{2+} and IP_3.

The third concept may help to clarify the origin of calcium waves and oscillations. The Ca^{2+} ion has a large mass and ionic radius and, in addition, its movements in the cytoplasm are obstructed by the tendency to form salts and complex compounds that bind it 99% [Prole 2019].
It was thought that IP_3 was highly mobile and, spreading rapidly in the cytosol, was responsible for the formation of calcium waves. The topic is under discussion, because experimental measurements seem to indicate the opposite, i.e. that IP_3 is not much more mobile than Ca^{2+} [Prole 2019].
On the other hand, the H^+ ion has a low mass and very small ionic radius and cannot be immobilized easily because it tends to give rise to soluble compounds. The discussion above, about the dissociation of IP_3, indicated the extremely high speed of the reactions freeing and consuming protons. Furthermore, the velocity of the protons in the cell has been measured and compared to the speed of sound [Fitchl 2016]. Locally generated protons are believed to be irrelevant, because cellular buffers can easily neutralize them. However, the neutralization process is not instantaneous [Donten 2012]. Experimental measurements of the diffusion coefficient of H^+ in the cytoplasm prove that, while neutralization occurs, protons are in motion and some of them can cover a significant distance [Al-Baldawi 1992, Swietach 2007]. Furthermore, they can modulate the action of enzymes, as suggested by Al-Baldawi et al. in the examples at the end of their publication [Al-Baldawi 1992]. So it can be inferred that at least part of these protons can activate other nearby acidifying enzymes and produce new Ca^{2+} spikes, in a repetitive manner.
Therefore, protons have all the chemical and physical characteristics required for them to be a basis for the formation of calcium waves and oscillations.

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5. CONTROL AND FEEDBACK

The various systems of calcium signaling are interconnected and control each other by the modulation of numerous Ca^{2+}-dependent enzyme activities including phospholipases, phosphatases, kinases, and phosphorylases, as well as the action of second messengers such as IP_3, cAMP and NAADP. It should be remembered that cAMP and NAADP, as well as cADPR, ATP, UTP, and S1P, can free protons similarly to IP_3 [Molinari 2015]. Furthermore, protons can mobilize other ions, such as Mg^{2+} and Zn^{2+} parallel to Ca^{2+} and these ionic cofactors can also specifically influence the activity of the enzymes involved.

The intensity of the stimulus induces diverse effects giving rise to both spatial and temporal modifications of the signal, with important functional consequences [Samanta 2017].

In these processes, the levels of ionic concentrations and particularly of $[Ca^{2+}]_i$ are a subtle discriminant. Generally there is a direct proportion between $[Ca^{2+}]_i$ and biological activity, but this is not always the case. In particular, IP_3Rs receptors are subject to biphasic regulation by $[Ca^{2+}]_i$, as mentioned above.

To protect itself from the risk that very rapid cellular biochemical processes, such as those involved in Ca^{2+}signaling, evade control, the organism carries out a number of different mechanisms.

For example, due to the immediate increase in PLC activity because of its Ca^{2+}-dependence, the chain reaction of the PLC/IP_3 pathway (the hydrolysis of PIP_2 – generation of IP_3 – liberation of protons – release in the cytosol of Ca^{2+} – the increase in Ca^{2+}-dependent phospholipase activity) could give rise to a self-sustained and self-stimulating closed circuit with the risk of an exponential increase in its activity were it not for control action with the opposite effect.

This action is carried out by phosphorylating enzymes, kinases and phosphorylases, which have the function of reconstructing the estero-phosphoric bindings previously split.

Consequently, the activation of phosphorylating enzymes also produced by the increase in $[Ca^{2+}]_i$ fosters biochemical processes that go in the opposite direction to the reactions of the catalyzed hydrolysis of the phospholipases and phosphatases as described above.

The action of phosphatidyl inositol phosphate kinase (PIPK) opposes that of PLC [Sun 2013], whilst the various kinases control the phosphatases by the synthesis of an array of inositol trisphosphate (IP_3) and tetrakisphosphate (IP_4) molecules [Irvine 2001, Shears 2004, Bunney 2010, Irvine 2016, Anguita 2018].

The opposing action must take place rapidly and with sufficient force, conditions guaranteed by the fact that phosphorylating enzymes are no less Ca^{2+}-dependent than the phospholipases and phosphatases.

Furthermore, for control to be effective, the opposing actions must comprise a balanced biochemical system, i.e. they must take place simultaneously. It has already been suggested that PIP_2 hydrolysis is stimulated concomitant with its synthesis [Loew 2007].

It could also be thought that the action of the phospholipases and phosphatases takes place alternately with that of the phosphorylating enzymes, in a rapid sequence of repeated hydrolysis and synthesis and that this could explain the appearance of the oscillations characteristic of calcium signaling [Salazar 2008, Vanderheyden 2009]. However, the phosphorylating enzymes act via chemical and biochemical mechanisms which are substantially different: the kinases transfer the phosphoric group of the ATP to the substrate, whilst the phosphorylases introduce the inorganic phosphoric group into the substrate.

To sustain alternance, it would be useful to show that the acidification produced by the hydrolysis is followed by alkalinization produced by the synthesis, i.e. prove that at least one of the two types of synthesis reaction, either the one catalyzed by the kinases (unlikely) or the one catalyzed by the phosphorylases (likely), is able to remove protons from the system and hence to alkalinize the cytosol.

In consideration of the above recalled H^+/Ca^{2+} interdependence, calcium waves and oscillations can be seen as consequence of parallel, underlying proton waves and oscillations; in other words $[Ca^{2+}]_i$

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always varies in space and time in parallel with $[H^+]_i$. Therefore, $[Ca^{2+}]_i$ can be influenced by either acidifying enzymes or alkalinizing enzymes or cytosolic buffers. An interesting question is: when acidification occurs, what is the prevalent opposing action, enzymatic alkalinization or cytosolic buffering? The topic deserves investigation and clarification.

6. THE CICR PHENOMENON

"Ca^{2+} Induced Ca^{2+} Release" (CICR) was discovered by studying the biochemical system of the cellular activity of the muscles [Ford 1970, Endo 1977, Endo 2009] and was defined as "*Ca^{2+} release by the action of Ca^{2+} alone without the simultaneous action of other activating processes*" [Endo 2009].

Therefore, it is the mechanism by which a small increase in cytosolic Ca^{2+} is able to induce the release of Ca^{2+} from the cellular stores of the endo/sarcoplasmic reticulum reaching significant $[Ca^{2+}]_i$ concentrations.

The cellular process of CICR has been confirmed experimentally on numerous occasions both for excitable and non-excitable cells. The phenomenon can occur by involvement of two types of receptors, IP_3Rs and ryanodine receptors (RyR) [Endo 2009, Laver 2018]. It is known that there is ~40% structural homology between IP_3R and RyR [Santulli 2017]. They are deputed to Ca^{2+} release and are activated through similar paths. It has been experimentally demonstrated that both the IP_3Rs and the RyRs can be activated by H^+ [Orchard 1987, Donoso 1996, Dabertrand 2011, Krizaj 2011, Hu 2017]. However, the molecular mechanism of the process by which CICR activates the gating of IP_3R and RyR channels has yet to be clarified.

The description of the metabolic phenomenon that occurs in the cellular cytoplasm is paradoxical from the biochemical point of view. It is known from the common ion effect that the addition of the same ion in a solution decreases its solubility. Therefore, the action described by the acronym CICR seems difficult to carry out from a purely ionic standpoint.

Nonetheless, given H^+/Ca^{2+} interdependence, there are two possible mechanisms for the gating of the IP_3R and RyR channels.

> **A -** In the simplest case, it could be thought that the small increase in the cytosolic Ca^{2+} activating the CICR is a consequence and indicator of a parallel increase in H^+, caused by the unconscious intervention of an operator, for example by introducing a buffer or reagent. Therefore, it is H^+ and not Ca^{2+} that causes the gating of the IP_3R and RyR channels. Since the mechanism is exclusively chemical, no other activating process is required.
>
> **B -** The second hypothesis presupposes the presence of an as yet undetected biological process that partially or totally modifies the cellular homeostasis of the calcium. For example, it could be thought that the small increase in cytosolic Ca^{2+} activates Ca-dependent phospholipases or phosphatases, which in turn produces the release of H^+ and hence the opening of the channels. Indeed, some authors have come to this conclusion to explain a phenomenon similar to CICR observed in smooth muscles [Ureña 2013].

The two hypotheses, A) and B) are not mutually exclusive and could occur simultaneously.

The expression "Ca^{2+} Induced Ca^{2+} Release" describes an important phenomenon without providing any explanation. Perhaps "Proton Induced Ca^{2+} Release" would be a more suitable expression.

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7. CONCLUSION

This work investigates some possible, important consequences of H^+/ Ca^{2+} interdependence in relation to calcium signaling. Given the chemical properties of these two ions and starting from the hypothesis that protons may have a stimulatory and not only regulatory role in the cell, it is possible to discuss the process in a new and broader manner and to posit biochemical explanations for the mechanisms causing some fundamental steps, which for some time have defied clarification. New connections can be suggested for the well-known events that occur, as established experimentally, in the process of calcium signaling, and a plausible and coherent picture can be built up for the basic mechanism involved.
Above all, it has been shown that the enzymatic activity of the phospholiphases and phosphatases frees protons. Investigations have been carried out into the ability of the protons to:

- generate calcium spikes and oscillations;
- trigger the gating of IP_3Rs and RyRs;
- induce CICR.

In our opinion these three important cytoplasmic activities carried out by the enzymatically produced protons are made possible by the particular chemical and physical characteristics of the protons themselves: very small, highly mobile, with a rapid reaction and ability to replace Ca^{2+} in its binding sites. These considerations are reason enough to posit the activation and not merely regulatory role of protons in calcium signaling. A significant role, that could be indispensable in two fundamental steps in the transduction of the signal: the release of bound Ca^{2+} and gating of IP_3R and RyR receptors. Therefore, it is important for the first time to present the basic events in the mechanism of calcium signaling within the general framework set out above.
The biochemical reactions involved in the production/consumption of protons inside the cell should be studied in depth. Among these, of particular interest are esterifications and the enzymes that catalyze them, in both directions, i.e. those of hydrolysis and synthesis. Enzymes such as phospholipases, phosphatases, kinases, and phosphorylases may significantly modify the levels of free calcium and hence all the spatial and temporal aspects of the related signal. About protons, it is important to confirm that the diffusion times are shorter than the neutralization times. Clearly, to carry out experiments into the processes in which protons play an important role, it is necessary to maintain the strictest control over pH and the buffering capacity of the materials under examination. Recent progress in calcium imaging techniques and particularly in biosensors, which allow to study subcellular H^+ and Ca^{2+} dynamics simultaneously, should bring to light the biochemical data and steps relating to the various processes and confirm the triggering role of protons set out above.

REFERENCES

Acuña-Castillo, C., Coddou, C., Bull, P., Brito, J. and Huidobro-Toro, J. P. (2007). Differential role of extracellular histidines in copper, zinc, magnesium and proton modulation of the P2X7 purinergic receptor. *Journal of Neurochemistry*, 101, 17–26. doi:10.1111/j.1471-4159.2006.04343.x

Al-Baldawi, N. F. and Abercrombie, R. F. (1992). Cytoplasmic hydrogen ion diffusion coefficient. *Biophysical Journal,* 61:1470-1479.

Alfonso, A., Cabado, A. G., Vieytes, M. R. and Botana, L. M. (2000). Calcium-pH crosstalks in rat mast cells: cytosolic alkalinization, but not intracellular calcium release, is a sufficient signal for degranulation. *British Journal of Pharmacology*, 130 (8): 1809-1816.

Anguita, E. and Villalobo, A. (2018). Ca^{2+} signaling and Src-kinases-controlled cellular functions. *Archives of Biochemistry and Biophysics*, 650: 59-74. doi: 10.1016/j.abb.2018.05.005.

Austin, C. and Wray, S. (2000). Interactions Between Ca^{2+} and H^{+} and Functional Consequences in Vascular Smooth Muscle. *Circulation Research*, 86 (3): 355-363.

Balla, T. (2013). Phosphoinositides: Tiny Lipids With Giant Impact on Cell Regulation. *Physiological Reviews*, 93 (3): 1019-1137.

Balnave, C. D. and Vaughan-Jones R. D. (2000). Effect of intracellular pH on spontaneous Ca^{2+} sparks in rat ventricular myocytes. *The Journal of Physiology*, 528 (1): 25-37.

Behera, S., Xu, Z., Luoni, L., Bonza, M. C., Doccula, F. G., De Michelis, M. I., Morris, R. J., Schwarzländer, M. and Costa, A. (2018). Cellular Ca^{2+} Signals Generate Defined pH Signatures in Plants. *The Plant Cell*, 30: 2704–2719.

Berdy, S. E., Kudla, J., Gruissem, W. and Gillaspy, G. E. (2001). Molecular Characterization of At5PTase1, an Inositol Phosphatase Capable of Terminating Inositol Trisphosphate Signaling. *Plant Physiology,* 126: 801-810.

Berggård, T., Silow, M., Thulin, E. and Linse, S. (2000). Ca2+- and H+-Dependent Conformational Changes of Calbindin D28k. *Biochemistry*, 39(23):6864-6873. https://doi.org/10.1021/bi992394g

Berra-Romani, R., Faris, P., Negri, S., Botta, L., Genova, T. and Moccia, F. (2019). Arachidonic Acid Evokes an Increase in Intracellular Ca^{2+} Concentration and Nitric Oxide Production in Endothelial Cells from Human Brain Microcirculation. *Cells*, 8: 689. doi:10.3390/cells8070689

Berridge, M. J. (2012). Calcium signalling remodelling and disease. *Biochemical Society Transactions*, 40 (2): 297-309.

Bezprozvanny, I., Watras, J. and Ehrlich, B. E. (1991). Bell-shaped calcium-response curves for Ins(1,4,5)P3- and calcium-gated channels from endoplasmic reticulum of cerebellum. *Nature*, 351: 751–754.

Bird, G. S. and Putney, W. J. jr. (2018). "Pharmacology of Store-Operated Calcium Entry Channels." In *Calcium Entry Channels in Non-Excitable Cells*, edited by Kozak, J. A. and Putney, J. W. jr. Methods in signal transduction series. Boca Raton (FL): CRC Press, Taylor & Francis Group, Chapter 16. doi: 10.1201/9781315152592-16

Bischof, H., Burgstaller, S., Waldeck-Weiermair, M., Rauter, T., Schinagl, M., Ramadani-Muja, J., Graier, W. F. and Malli, R. (2019). Live-Cell Imaging of Physiologically Relevant Metal Ions Using Genetically Encoded FRET-Based Probes. *Cells*, 8 (5): E492.

Bootman, M. D., Allman, S., Rietdorf, K. and Bultynck, G. (2018). Deleterious effects of calcium indicators within cells; an inconvenient truth. *Cell Calcium*, 73: 82-87.

Bootman, M. D. and Bultynck, G. (2019). Fundamentals of Cellular Calcium Signaling: A Primer. *Cold Spring Harbor Perspectives in Biology*, doi: 10.1101/cshperspect.a038802.

Brini, M. and Carafoli, E. (2011). The plasma membrane Ca^{2+} ATPase and the plasma membrane sodium calcium exchanger cooperate in the regulation of cell calcium. *Cold Spring Harbor Perspectives in Biology*, 3 (2): a004168. doi: 10.1101/cshperspect.a004168.

Bui, Y. K. and Sternberg, P. W. (2002). *Caenorhabditis elegans* Inositol 5-Phosphatase Homolog Negatively Regulates Inositol 1,4,5-Triphosphate Signaling in Ovulation. *Molecular Biology of the Cell*, 13: 1641–1651.

Bunney, T. D. and Katan, M. (2010). Phosphoinositide signalling in cancer: beyond PI3K and PTEN. *Nature Reviews Cancer*, 10 (5): 342-52.

Busa, W. B. and Nuccitelli, R. (1984). Metabolic regulation via intracellular pH. *American Journal of Physiology*. 246 (4 Pt 2): R409-38. https://doi.org/10.1152/ajpregu.1984.246.4.R409.

Cairns, S.P., Westerblad, H. and Allen, D. G. (1993). Changes in myoplasmic pH and calcium concentration during exposure to lactate in isolated rat ventricular Myocytes. *Journal of Physiology*, 464: 561-574.

Carafoli, E. and Krebs, J. 2016. Why calcium? How Calcium Became the Best Communicator. *The Journal of Biological Chemistry*, 291 (40): 20849-20857.

Chandran, A., Chee, X., Prole, D. L. and Rahman, T. (2019). Exploration of inositol 1,4,5-trisphosphate (IP3) regulated dynamics of N-terminal domain of IP_3 receptor reveals early phase molecular events during receptor activation. *Scientific Reports*, 9 (1): 2454.

Chen, W-H., Chen, C-H., Yang, K-T., Chang, W-L., Su, M-J., Wu, C-C. and Wu, M-W. (2001). Arachidonic acid-induced H^+ and Ca^{2+} increases in both the cytoplasm and nucleoplasm of rat cerebellar granule cells. *The Journal of Physiology*, 537 (2): 497–510.

Clapham, D. E. (2007). Calcium signaling. *Cell,* 131 (6): 1047-58.

Cocco, L., Follo, M., Manzoli, L. and Suh P-G. (2015). Phosphoinositide-specific phospholipase C in health and disease. *Journal of Lipid Research*, 56 (10): 1853-1860.

Colina-Tenorio, L., Dautant, A., Miranda-Astudillo H., Giraud, M. F. and Gonzàlez-Halphen, D. (2018). The peripheral Stalk of Rotary ATPases. *Frontiers in Physiology*, 9: 1243.

Costa, A., Navazio, L. and Szabo, I. (2018). The contribution of organelles to plant intracellular calcium signaling. *Journal of Experimental Botany*, 69(17):4175–4193. doi:10.1093/jxb/ery185.

Dabertrand, F., Nelson, M. T. and Brayden, J. E. (2011). Acidosis Dilates Brain Parenchymal Arterioles by Conversion of Calcium Waves to Sparks to Activate BK Channels. *Circulation Research,* 110(2): 285-94. doi: 10.1161/CIRCRESAHA.111.258145

Daugirdas, J. T., Arrieta, X., Ye, M., Flores, G. and Batile, D. C. (1995). Intracellular Acidification Associated with Changes in Free Cytosolic Calcium. Evidence for Ca^{2+}/H^{+} Exchange via a Plasma Membrane Ca^{2+}-ATPase in Vascular Smooth Muscle Cells. *The Journal of Clinical Investigation*, 95 (4): 1480-1489.

DeCoursey, T. E. (2018). Gating currents indicate complex gating of voltage-gated proton channels. *Proceedings of the National Academy of Science*, 115 (37): 9057-9059. https://doi.org/10.1073/pnas.1813013115

Depaoli, M. G., Bischof, H., Eroglu, E., Burgstaller, S., Ramadani-Muja, J., Rauter, T., Schinagl, M. et al. (2019). Live cell imaging of signaling and metabolic activities. *Pharmacology &Therapeutics*, Jun 7. pii: S0163-7258(19)30110-X. https://doi.org/10.1016/j.pharmthera.2019.06.003

Dong, R., Zhu, T., Benedetti, L., et al. (2018). The inositol 5-phosphatase INPP5K participates in the fine control of ER organization. *The Journal of Cell Biology*, 217(10):3577-3592.

Donoso, P., Beltrán, M., and Hidalgo, C. (1996). Luminal pH Regulates Calcium Release Kinetics in Sarcoplasmic Reticulum Vesicles. *Biochemistry*, 35 (41): 13419–13425.

Donten, M. L., Vandevondele, J. and Hamm, P. (2012). Speed limits for acid-base chemistry in aqueous solutions. *Chimia International Journal for Chemistry*, 66 (4): 182-186.

Dubois, I. and Gerday, C. 1990. Soluble calcium-binding proteins: parvalbumins and calmodulin from eel skeletal muscle. *Comparative Biochemistry and Physiology B*, 95 (2): 381-5.

Dupont, G., Combettes, L., Bird, G. S., and Putney, J. W. (2011) Calcium Oscillations. *Cold Spring Harbor Perspectives in Biology*, 3 (3): a004226.

Dyson, J. M., Fedele, C. G., Davies, E. M., Becanovic, J. and Mitchell, C. A. (2012). Phosphoinositide phosphatases: just as important as the kinases. *Subcellular Biochemistry,* 58:215-79. doi: 10.1007/978-94-007-3012-0_7.

Endo, M. (1977). Calcium release from the sarcoplasmic reticulum. *Physiological Reviews*, 57: 71–108.

Endo, M. (2009). Calcium-Induced Calcium Release in Skeletal Muscle. *Physiological Reviews*, 89: 1153–1176.

Fan, J.-S., Goh, H., Ding, K., Xue, B., Robinson, R. C. and Yang, D. (2017). Structural Basis for pH-mediated Regulation of F-actin Severing by Gelsolin Domain. *Scientific Reports*, 7:45230.

Faris, P., Shekha, M., Montagna, D., Guerra, G. and Moccia, F. (2019). Endolysosomal Ca^{2+} Signalling and Cancer Hallmarks: Two-Pore Channels on the Move, TRPML1 Lags Behind! *Cancers*, 11, 27.

Felle, H. H. (2001). pH: Signal and Messenger in Plant Cells. *Plant Biology*, 3(6):577-591.

Fichtl, B., Shrivastava, S. and Schneider, M. (2016). Protons at the speed of sound: Predicting specific biological signaling from physics. *Scientific Reports*, 6, 22874. https://doi.org/10.1038/srep22874

Fogarc, M. (2007). Vacuolar ATPases: rotary proton pumps in physiology and pathophysiology. *Nature Reviews Molecular Cell Biology*, 8 (11): 917-929.

Ford, L.E. and Podolsky, R.J. (1970). Regenerative calcium release within muscle cells. *Science*, 167: 58–59.

Foskett, J. K., White, C., Cheung, K. H. and Mak, D.O. (2007). Inositol trisphosphate receptor Ca^{2+} release channels. *Physiological Reviews*, 87: 593–658.

Galione, A. (2019). NAADP Receptors. *Cold Spring Harbor Perspectives in Biology*, pii: a035071. doi: 10.1101/cshperspect.a035071.

Gaspers, L. D., Bartlett, P. J., Burnett, P., Metzger, W., Johnston, J., Joseph, S. K., Höfer, T. and Thomas, A. P. (2014). Hormone-induced calcium oscillations depend on cross-coupling with inositol 1,4,5-trisphosphate oscillations. *Cell Reports*, 9: 1209–18.

González, A., Pfeiffer, F., Schmid, A., and Schulz, I. (1998). Effect of intracellular pH on acetylcholine-induced Ca^{2+} waves in mouse pancreatic acinar cells. *American Journal of Physiology Cell Physiology*, 275: C810-C817.

Goss, F., Prushko, J. and Bogner, R. (2010). Factors affecting calcium precipitation during neutralisation in a simulated intestinal environment. *Journal of Pharmaceutical Science*, 99 (10): 4183-91.

Greotti, E. and Pozzan, T. (2020). Live Mitochondrial or Cytosolic Calcium Imaging Using Genetically-encoded Cameleon Indicator in Mammalian Cells. *Bio-Protocol* 10(3): e3504. doi: 10.21769/BioProtoc.3504.

Gurung, R., Tan, A., Ooms, L. M., et al. (2003). Identification of a novel domain in two mammalian inositol-polyphosphate 5-phosphatases that mediates membrane ruffle localization. The inositol 5-phosphatase skip localizes to the endoplasmic reticulum and translocates to membrane ruffles following epidermal growth factor stimulation. *The Journal of Biological Chemistry,* 278(13): 11376-85.

Guse, A. H., Roth, E. and Emmrich, F. (1994). Ca^{2+} release and Ca^{2+} entry induced by rapid cytosolic alkalinization in Jurkat T-lymphocytes. *Biochemical Journal*, 301(1): 83-88.

Hamada, K. and Mikoshiba, K. (2020). IP_3 Receptor Plasticity Underlying Diverse Functions. *Annual Review of Physiology,* 82:151-86. https://doi.org/10.1146/annurev-physiol-021119-034433

Hegedűs, L. et al. (2020). Molecular Diversity of Plasma Membrane Ca2+ Transporting ATPases: Their Function Under Normal and Pathological Conditions. In: Islam M. (eds) Calcium Signaling. *Advances in Experimental Medicine and Biology*, vol 1131. Springer, Cham

Hincke, M. T., McCubbin, W. D. and Kay, C. M. (1978). Calcium-binding properties of cardiac and skeletal troponin C as determined by circular dichroism and ultraviolet difference spectroscopy, *Canadian Journal of Biochemistry*, 56 (178): 384-95.

Hsu, FS. and Mao, Y. (2015). The structure of phosphoinositide phosphatases: Insights into substrate specificity and catalysis. *Biochimica et Biophysica Acta*, 1851 (6): 698–710.

Hu,Y.-L., Mi, X., Huang, C., Wang, H.-F., Song, J.-R., Shu, Q., Ni, L., Chen, J.-G., Wang, F. and Hu, Z.-L. (2017). Multiple H^+ sensors mediate the extracellular acidification-induced $[Ca^{2+}]_i$ elevation in cultured rat ventricular cardiomyocytes. *Scientific Reports,* 7, 44951. https://doi.org/10.1038/srep44951.

Huang, J., Liu, C. H., Hughes, S. A., Postma, M., Schwiening, C. J. and Hardie, R. C. (2010). Activation of TRP channels by protons and phosphoinositide depletion in Drosophila photoreceptors. *Current Biology*, 20(3):189–197.

Iida, S. and Potter, J. D. (1986). Calcium binding to calmodulin. Cooperativity of the calcium-binding sites. *The Journal of Biochemistry*, 99(6):1765-1772. https://doi.org/10.1093/oxfordjournals.jbchem.a135654.

Irvine, R. F. (2016). A short history of inositol lipids. In Thematic Review Series: Living History of Lipids. *Journal of Lipid Research*, 57 (11): 1987-1994.

Irvine, R. F., and Schell, M. J. (2001). Back in the water: the return of the inositol phosphates. *Nature Reviews Molecular Cell Biology*, 2: 327–338.

Ijuin, T., Mochizuki, Y., Fukami K., Funaki M., Asano T., and Takenawa, T. (2000). Identification and characterization of a novel inositol polyphosphate 5-phosphatase. *The Journal of Biological Chemistry,* 275:10870-10875.

Jefferson, A. B. and Majerus, P. W. (1995). Properties of type II inositol polyphosphate 5-phosphatase. *The Journal of Biological Chemistry,* 270(16): 9370-7.

Jia, Q., Kong, D., Li, Q., Sun, S.,Song, J., Zhu, Y., Liang, K., Ke, Q., Lin W., and Huang, J. (2019). The Function of Inositol Phosphatases in Plant Tolerance to Abiotic Stress. *International Journal of Molecular Sciences,* 20, 3999. doi:10.3390/ijms20163999.

Johenning, F. W., Wenk, M. R., Uhlén, P., Degray, B., Lee, E., De Camilli, P. and Ehrlich, B. E. (2004). $InsP_3$-mediated intracellular calcium signalling is altered by expression of synaptojanin-1. *Biochemical Journal,* 382: 687-694.

Kanzaki, Y., Tokuda, K. and Bruckensteinc, S. (2014). Dissociation Rates of Weak Acids Using Sinusoidal Hydrodynamic Modulated Rotating Disk Electrode Employing Koutecky-Levich Equation. *Journal of The Electrochemical Society*, 161 (12): H770-H779.

Kiang, J. G. (1991). Effect of intracellular pH on cytosolic free [Ca^{2+}] in human epidermoid A-431 cells. *European Journal of Pharmacology: Molecular Pharmacology*, 207 (4): 287-296.

Kim, D. and Smith, T. W. (1988). Cellular mechanisms underlying calcium-proton interactions in cultured chick ventricular cells. *Journal of Physiology*, 398: 391-410.

Kintzer, A. F., Stroud, R. M. (2018). On the structure and mechanism of two-pore channels. *The FEBS Journal*, 285(2):233-243.

Kozak, J.A. and Putney, J.W. jr. editors. (2017). *Calcium Entry Channels in Non-Excitable Cells.* Methods in signal transduction series. Boca Raton (FL): CRC Press.

Krizaj, D., Mercer, A. J., Thoreson, W.B. and Barabas, P. (2011). Intracellular pH modulates inner segment calcium homeostasis in vertebrate photoreceptors. *American Journal of Physiology Cell Physiology*, 300 (1): C187-C197.

Laver, D. R. (2018). Regulation of the RyR channel gating by Ca^{2+} and Mg^{2+}. *Biophysical Reviews*, 10:1087–1095. https://doi.org/10.1007/s12551-018-0433-4.

Laxminarayan, K. M., Matzaris, M., Speed, C. J., and Mitchell, C. A. (1993). Purification and characterization of a 43-kDa membrane-associated inositol polyphosphate 5-phosphatase from human placenta. *The Journal of Biological Chemistry*, 268(7), 4968–4974.

Li, S., Hao, B., Lu, Y., Yu, P., Lee, H. C. and Yue, C. (2012). Intracellular Alkalinization Induces Cytosolic Ca^{2+} Increases by Inhibiting Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA). *PloS One*, 7 (2): e31905.

Lindeman, K. S., Croxton, T. L., Lande, B. and Hirshman, C. A. (1998). Hypocapnia-induced contraction of porcine airway smooth muscle. *European Respiratory Journal*, 12 (5): 1046–1052.

Liu, Z., Pei, H., Zhang, L. and Tian, Y. (2018). Mitochondria-Targeted DNA Nanoprobe for Real-Time Imaging and Simultaneous Quantification of Ca2+ and pH in Neurons. *ACS Nano,* 26;12(12): 12357-12368. doi: 10.1021/acsnano.8b06322.

Liu, Q., Huang, S., Yin, P., Yang, S., Zhang, J., Jing, L., Cheng, S., Tang, B., Li, X.-J., Pan, Y. and Li, S. (2020). Cerebellum-enriched protein INPP5A contributes to selective neuropathology in mouse model of spinocerebellar ataxias type 17. *Nature Communications,* 11:1101. https://doi.org/10.1038/s41467-020-14931-8.

Loew, L. M. (2007). Where does all the PIP_2 come from? *The Journal of Physiology*, 582 (3): 945-951.

Lynch, B. J., Muqit, M. M. K., Walker, T. R. and Chilvers, E. R. (1997). [3H]Inositol Polyphosphate Metabolism in Muscarinic Cholinoceptor-Stimulated Airways Smooth Muscle: Accumulation of [3H]inositol 4,5 Bisphosphate *Via* a Lithium-Sensitive Inositol Polyphosphate 1-Phosphatase. *Journal of Pharmacology and Experimental Therapeutics*, 280 (2) 974-982.

MacConaill, M. (1985). Calcium precipitation from mammalian physiological salines (Ringer solutions) and the preparation of high [Ca] media. *Journal of Pharmacological Methods*, 14 (2): 147-55. https://doi.org/10.1016/0160-5402(85)90052-X

Majerus, P. W. (1992). Inositol phosphate biochemistry. *Annual Review of Biochemistry*, 61: 225-250.

Majerus, P. W., Kisseleva, M. V. and Norris, F. A. The Role of Phosphatases in Inositol Signaling Reactions. *The Journal of Biological Chemistry*, 1999, 274, 10669–10672.

Marin, M., Sellier, C., Paul-Antoine, A. F., Cailliau, K., Browaeys-Poly, E., Bodart, J. F. and Vilain, J. P. (2010). Calcium Dynamics During Physiological Acidification in *Xenopus* Oocyte. *The Journal of Membrane Biology*, 236 (3): 233–245.

McCudden, C. R. (2013). pH-adjusted ionized calcium. Accessed August 4, 2019. Article downloadable from acutecaretesting.org. https://acutecaretesting.org/en/articles/ph-adjusted-ionized-calcium.

McPherson, P. S., Garcia, E. P. , Slepnev, V. I., David, C., Zhang, X., Grabs, D., Sossin, W.S., Bauerfeind, R., Nemoto, Y., De Camilli, P. (1996). A presynaptic inositol-5-phosphatase. *Nature*. 379:353–357.

Metha, Z. B., Pietka, G. and Lowe, M. (2014). The Cellular and Physiological Functions of Lowe Syndrome Protein OCRL1. *Traffic,* 15: 471-487.

Michell, R. H., Kirk, C. J., Jones, L. M., Downes, C. P. and Creba, J. A. (1981). The stimulation of inositol lipid metabolism that accompanies calcium mobilization in stimulated cells: defined characteristics and unanswered questions. *Philosophical Transactions of the Royal Society B*, 296 (1080): 123-137. doi 10.1098/rstb.1981.0177.

Minelli, A., Lyons, S., Nolte, C., Verkhratsky, A. and Kettenmann, H. (2000). Ammonium triggers calcium elevation in cultured mouse microglial cells by initiating Ca(2+) release from thapsigargin-sensitive intracellular stores. *Pflügers Archiv: European Journal of Physiology*, 439 (3): 370-7.

Mitchell, C. A., Gurung, R., Kong, A. M., Dyson,J. M., Tan, A. and Lisa M. Ooms. (2002). Inositol Polyphosphate 5-Phosphatases: Lipid Phosphatases With Flair. *IUBMB Life*, 53: 25-36

Mochizuki, Y. and Takenawa, T. (1999). Novel Inositol Polyphosphate 5-Phosphatase Localizes at Membrane Ruffles. *The Journal of Biological Chemistry*, 274(51): 36790–36795.

Molinari, G. (2015). Is hydrogen ion (H(+)) the real second messenger in calcium signalling? *Cellular Signalling*, 27 (7): 1392-7.

Molinari, G. and Nervo, E. (2019). Phospholipases cause transient increases of H^+ and Ca^{2+} concentrations. [version 1; not peer reviewed]. *F1000Research*, 8:270 (poster) https://doi.org/10.7490/f1000research.1116471.1

Nagaoka, T., Masuda, A. and Takamatsu, T. (1997). Lowering Extracellular pH Raises Intracellular Calcium in Cultured Rat Astrocytes. *Acta Histochemica et Cytochemica*, 30 (2): 221-225.

Nakamura, Y. and Kiyoko, F. (2017). Regulation and physiological functions of mammalian phospholipase C. *Journal of Biochemistry*, 161 (4): 315–321.

Nishiguchi, H., Hayashi, T., Shigetomi, T., Ueda, M. and Tomita, T. (1997). Changes in Intracellular Ca^{2+} Concentration Produced by the Alteration of Intracellular pH in Rat Parotid Acinar Cells. *Japanese Journal of Physiology*, 47: 41-49.

Nitschke, R., Riedel, A., Ricken, S., Leipziger, J., Benning, N., Fischer, K. G. and Greger, R. (1996). The effect of intracellular pH on cytosolic Ca^{2+} in HT29 cells. *Pflügers Archiv: European Journal of Physiology*, 433 (1-2): 98-108.

Onufriev, A. V. and Alexov, E. (2013). Protonation and pK changes in protein-ligand binding. *Quarterly Reviews of Biophysics*, 46(2): 181–209. doi:10.1017/S0033583513000024.

Orchard, C. H., Houser, S.R., Kort, A.A., Bahinski, A., Capogrossi, M. C. and Lakatta, E. G. (1987). Acidosis Facilitates Spontaneous Sarcoplasmic Reticulum Ca^{2+} Release in Rat Myocardium. *Journal of General Physiology*, 90 (1): 145-165.

Paillamanque, J., Madrid, C., Carmona, E. M., Osses, N., Moreno, R. D., Oresti, G. M., Pino, J. A. and Reyes, J. G. (2016). Effects of Fatty Acids on Intracellular [Ca^{2+}], Mitochondrial Uncoupling and Apoptosis in Rat Pachytene Spermatocytes and Round Spermatids. *PloS One*, 11 (7): e0158518.

Pallafacchina, G., Zanin, S. and Rizzuto, R. (2018). Recent advances in the molecular mechanism of mitochondrial calcium uptake. [version 1; peer review: 4 approved] *F1000Research 2018*, 7 (F1000 Faculty Rev): 1858. https://doi.org/10.12688/f1000research.15723.1

Pattni, K. and Banting, G. (2004). Ins(1,4,5)P3 metabolism and the family of IP3-3Kinases. *Cellular Signalling,* 16(6):643-54.

Periodic table of elements org. n.d. "Solubility table." Accessed August 4, 2019. https://periodic-table-of-elements.org/SOLUBILITY.

Poburko, D., Santo-Domingo, J., and Demaurex, N. (2011). Dynamic Regulation of the Mitochondrial Proton Gradient during Cytosolic Calcium Elevations. *The Journal of Biological Chemistry*, 286 (13): 11672–11684.

Pozzan, T., Rizzuto, R., Volpe, P. and Meldolesi, J. (1994). Molecular and cellular physiology of intracellular calcium stores. *Physiological Reviews*, 74 (3): 595-636.

Primeau, J. O., Armanious, G. P., Fisher, M. E. and Young, H. S. (2018). The SarcoEndoplasmic Reticulum Calcium ATPase. *Subcellular Biochemistry,* 87: 229-258.

Prole, D.L. and Taylor, C.W. (2019). Structure and Function of IP_3 Receptors. *Cold Spring Harbor Perspectives in Biology*, 11 (4). pii: a035063. doi: 10.1101/cshperspect.a035063.

Raffaello, A., Mammucari, C., Gherardi, G., and Rizzuto, R. (2016). Calcium at the center of cell signaling: interplay between endoplasmic reticulum, mitochondria and lysosomes. *Trends in Biochemical Science*, 41 (12): 1035–1049.

Ramos, A. R., Ghosh, S. and Erneux, C. (2019). The impact of phosphoinositide 5-phosphatases on phosphoinositides in cell function and human disease. *The Journal of Lipid Research,* 60: 276-286.

Ribeiro, D., Freitas, M., Rocha, S., Lima, J. L. F. C., Carvalho, F. and Fernandes, E. (2018). Calcium Pathways in Human Neutrophils-The Extended Effects of Thapsigargin and ML-9. *Cells*, 7 (11): E204.

Rose, H. J., Dargan, S., Shuai, J. and Parker, I. (2006). 'Trigger' Events Precede Calcium Puffs in Xenopus Oocytes. *Biophysical Journal*, 91: 4024–4032.

Rossi, A.M., Taylor, C.W. (2018). IP_3 receptors – lessons from analyses ex cellula. *Journal of Cell Science*, 132: jcs222463. doi: 10.1242/jcs.222463.

Roth-Bassel, H. A. and Clydesdale, F. M. (1992). In Vitro Solubility Characteristics of Six Calcium Salts. *Journal of Food Protection*, 55 (12): 1003-1005. https://doi.org/10.4315/0362-028X-55.12.1003.

Rudge, S. A. and Wakelam, M. J. O. (2016). Phosphatidylinositolphosphate phosphatase activities and cancer. *Journal of Lipid Research*, 57: 176-192.

Salazar, C., Politi, A. Z. and Höfer, T. (2008). Decoding of calcium oscillations by phosphorylation cycles: analytic results. *Biophysical Journal*, 94 (4): 1203-15.

Samanta, K. and Parekh, A. B. (2017). Spatial Ca^{2+} profiling: decrypting the universal cytosolic Ca^{2+} oscillation. *The Journal of Physiology*, 595: 3053–3062.

Santulli, G., Nakashima, R., Yuan, Q. and Andrew R. Marks (2017). Intracellular calcium release channels: an update. *The Journal of Physiology,* 595(10): 3041–3051.

Schmid, A. C., Wise, H. M., Mitchell, C. A., Nussbaum, R. and Woscholki, R. (2004). Type II phosphoinositide 5-phosphatases have unique sensitivities towards fatty acid composition and head group phosphorylation. *FEBS Letters,* 576: 9-13.

Shears, S. B. (2004). How versatile are inositol phosphate kinases? *Biochemical Journal*, 377: 265-280.

Siciliano, C. A. and Tye, K. M. (2019). Leveraging calcium imaging to illuminate circuit dysfunction in addiction. *Alcohol*, 74: 47-63.

Sims, C. E. and Allbritton, N. L. (1998). Metabolism of inositol 1,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate by the oocytes of Xenopus laevis. *The Journal of Biological Chemistry*, 273 (7): 4052-8.

Smith, N. A., Kress, B. T., Lu, Y., Chandler-Militello, D., Benraiss, A. and Nedergaard, M. (2018). Fluorescent Ca^{2+} indicators directly inhibit the Na,K-ATPase and disrupt cellular functions. *Science Signaling*, 11 (515): eaal2039. doi: 10.1126/scisignal.aal2039.

Speed, C. J., Matzaris, M., Bird, P. I. and Mitchell, C. (1995). Tissue distribution and intracellular localisation of the 75-kDa inositol polyphosphate 5-phosphatase. *European Journal of Biochemistry*, 234: 216-224

Stewart, A. G., Laming, E. M., Sobti, M. and Stock D. (2014). Rotary ATPases - dynamic molecular machines. *Current Opinion in Structural Biology*, 25: 40-48.

Streb, H., Irvine, R.F., Berridge, M.J. and Schulz, I. (1983). Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. *Nature*, 306 (5938): 67-9.

Sun, Y., Thapa, N., Hedman, A. C. and Anderson, R. A. (2013). Phosphatidylinositol-4,5-bisphosphate: Targeted Production and Signaling. *Bioessays*, 35 (6): 10.1002/bies.201200171.

Swietach, P., Spitzer, K. W. and Vaughan-Jones, R. D. (2007). pH-Dependence of Extrinsic and Intrinsic H^+-Ion Mobility in the Rat Ventricular Myocite, Investigated Using Flash Photolysis of a Caged-H^+ Compound. *Biophysical Journal,* 92(2):641-653.

Thillaiappan, N. B., Chavda, A. P., Tovey, S. C., Prole, D. L. and Taylor, C. W. (2017). Ca^{2+} signals initiate at immobile IP3 receptors adjacent to ER-plasma membrane junctions. *Nature Communications*, 8(1): 1505. doi: 10.1038/s41467-017-01644-8.

Thomas, R. C. (2002). The Effects of HCl and $CaCl_2$ Injections on Intracellular Calcium and pH in Voltage-clamped Snail (*Helix aspersa*) Neurons. *Journal of General Physiology*, 120 (4): 567–579. doi: 10.1085/jgp.20028665

Ureña, J., Fernández-Tenorio, M., Porras-Gonzáles, C., González-Rodríguez, P., Castellano, A. and López-Barneo, J. (2013). A new metabotropic role for L-type Ca(2+) channels in vascular smooth muscle contraction. *Current Vascular Pharmacology*, 11 (4): 490-6.

Vanderheyden, V., Devogelaere, B., Missianen, L., De Smedt, H., Bultynck, G. and Parys, J. B. (2009). Regulation of inositol 1,4,5-trisphosphate-induced Ca^{2+} release by reversible phosphorylation and dephosphorylation. *Biochimica et Biophysica Acta*, 1793 (6): 959–970.

Vangeel, L. and Voets, T. (2019). Transient Receptor Potential (TRP) Channels and Calcium Signaling. *Cold Spring Harbor Perspectives in Biology*, 11 (6): a035048.

Wilson, C., Lee, M. D., Heathcote, H. R., Zhang, X., Buckley, C., Girkin J. M., Saunter, C. D., and McCarron, J. G. (2019). Mitochondrial ATP production provides long-range control of endothelial inositol trisphosphate–evoked calcium signaling. *The Journal of Biological Chemistry*, 294 (3): 737–758.

Worley, P. F., Baraban, J. M., Supattapone, S., Wilson, V. S. and Snyder, S. H. (1987). Characterization of inositol trisphosphate receptor binding in brain. Regulation by pH and calcium. *The Journal of Biological Chemistry*, 262: 12132–12136.

Xu, H., Martinoia, E. and Szabo, I. (2015). Organellar Channels and Transporters. *Cell Calcium*, 58 (1): 1–10. https://doi.org/10.1016/j.ceca.2015.02.006.

Yang, J., Zhao, Z., Gu, M., Feng, X. and Xu, X. (2019). Release and uptake mechanisms of vesicular Ca^{2+} stores. *Protein & Cell*, 10 (1): 8–19. https://doi.org/10.1007/s13238-018-0523-x

Yang, Y., Liu, N., He, Y., Liu, Y., Ge, L., Zou, L., Song, S., Xiong, W. and Liu, X. (2018). Improved calcium sensor GCaMP-X overcomes the calcium channel perturbations induced by the calmodulin in GcaMP. *Nature Communications*, 9 (1): 1504. doi: 10.1038/s41467-018-03719-6.

Yoon, S. Y. (2019). Role of Type 1 Inositol 1,4,5-triphosphate Receptors in Mammalian Oocytes. *Development & Reproduction*, 23 (1): 1-9.

Yu, A. S., Yue, Z., Feng, J. and Yue, L. (2018). "Regulation of Orai/STIM Channels by pH." In *Calcium Entry Channels in Non-Excitable Cells*, edited by Kozak, J. A., and Putney, J. W. jr. Methods in signal transduction series. Boca Raton (FL): CRC Press, Taylor & Francis Group, 9: 161-176.

Zhang, J.-B., Chang, S., Xu, P.,….,Luo, C., Zhang, X. and Zhu, S. (2018). Structural Basis of the Proton Sensitivity of Human GluN1-GluN2A NMDA Receptors. *Cell Reports,* 25: 3582-3590.

Zhang, X., Jefferson, A. B., Auethavekiat, V. and Majerus, P. W. (1995). The protein deficient in Lowe syndrome is a phosphatidylinositol-4,5-bisphosphate 5-phosphatase. *Proceedings of the National Academy of Sciences U.S.A.,* 92:4853-4856.