Ca^{2+} Signaling in Exocrine Cells

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Calcium (Ca^{2+}) and cyclic AMP (cAMP) signaling cross talk and synergize to stimulate the cardinal functions of exocrine cells, regulated exocytosis, and fluid and electrolyte secretion. This physiological process requires the organization of the two signaling pathways into complexes at defined cellular domains and close placement. Such domains are formed by membrane contact sites (MCS). This review discusses the basic properties of Ca^{2+} signaling in exocrine cells, the role of MCS in the organization of cell signaling and in cross talk and synergism between the Ca^{2+} and cAMP signaling pathways and, finally, the mechanism by which the Ca^{2+} and cAMP pathways synergize to stimulate epithelial fluid and electrolyte secretion.

Calcium (Ca^{2+}) signaling synergizes with other signaling pathways, in particular the cyclic AMP (cAMP) pathway, to mediate numerous cellular functions, including the cardinal functions of exocrine cells, regulated exocytosis, and fluid and electrolyte secretion (Lee et al. 2012). Both functions are vectorial and polarized and thus the signals that regulate them must be generated and function in a polarized and vectorial manner. Moreover, intracellular organelles, mitochondria, lysosomes, endosomes, and secretory granules shape the transduction signals and control the signal localization, propagation pattern, and distance (Raffaello et al. 2016). To achieve this form of communication, signaling proteins and cellular organelles are expressed in a highly polarized manner and signaling proteins are organized in specific cellular microdomains. These microdomains ensure the specificity of signaling to meet physiological needs and allow communication and transfer of material and molecules between organelles.

Each signaling pathway includes multiple proteins with defined function. In the complexes, the proteins are in molecular distances with respect to each other. Compartmentalized signaling complexes are found in the plasma membrane (PM), the endoplasmic reticulum (ER), endosomes, lysosomes, mitochondria, and the nuclear envelope. A significantly improved molecular understanding of cell signaling and communication within signaling pathways emerged with the improved understanding of formation and function of membrane contact sites (MCS). MCS are formed by the ER, which spans the entire cell cytosol, and the membranes of all other cellular membranes. MCS are formed by tethering proteins that form a bridge between the PM and the ER to form the ER/PM junctions (Cao et al. 2015; Henne et al. 2015). There are

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specific tether proteins that form MCS between the ER/mitochondria (Westermann 2015), ER/endosomes, ER/lysosomes (Penny et al. 2015), ER/Golgi (Mesmin et al. 2019), and ER/nuclear membrane (Prinz 2014). Many MCS serve to communicate and transfer material between membranes, particularly lipids and signaling molecules (Ca^{2+}, cAMP, and possibly others) (Lahiri et al. 2015). In this review, we focus on cell signaling in exocrine glands and the significance of MCS, in particular the ER/PM junction, in exocrine cell signaling. The role of other MCS in cell signaling can be found in Prinz (2014), Penny et al. (2015), Muallem et al. (2017), Tepikin (2018), and Wu et al. (2018).

BASIC PROPERTIES OF Ca^{2+} SIGNALING IN EXOCRINE CELLS

The receptor-evoked Ca^{2+} signal is dependent on a combination of Ca^{2+} pumps that generate Ca^{2+} gradients across the PM, the ER membrane, and the membrane of all other cellular organelles. The PM Ca^{2+} ATPase (PMCA) pump (Calì et al. 2017) is the primary Ca^{2+} extruding mechanism at the PM that forms a 10^4-fold Ca^{2+} gradient between the cytoplasm and the cell exterior. The sarco-ER ATPase (SERCA) pump (Chemaly et al. 2018) mediates Ca^{2+} uptake into the ER to form about a 5×103-fold gradient between the ER and the cytoplasm. The secretory pathway Ca^{2+} ATPase (SPCA) pumps mainly pump Ca^{2+} into the Golgi (Dang and Rao 2016). Ca^{2+} uptake into the mitochondria is driven by the mitochondrial membrane potential and is mediated by the mitochondrial Ca^{2+} uniporter (MCU) complex that includes the pore forming MCU, the gatekeepers MICU1 and MICU2, and the scaffold EMRE (Nemani et al. 2018). The mechanism mediating Ca^{2+} uptake into lysosomes, endosomes, and secretory granules is not well understood, although a recent study suggested that Ca^{2+} released from the ER at ER/lysosomes MCS feeds the lysosomes with Ca^{2+} that is taken into the lysosome by an unknown pathway (Garrity et al. 2016). On the other hand, a transporter proposed to function as Ca^{2+}/H^+ exchanger named CAX is expressed in acidic organelles and affect their Ca^{2+} content, which may be the major Ca^{2+} uptake mechanism in these organelles and take up Ca^{2+} released by the ER (Melchionda et al. 2016).

Two Ca^{2+} sources contribute to the receptor-mediated increase in cytoplasmic Ca^{2+}: Ca^{2+} release from intracellular stores and Ca^{2+} influx from the extracellular space into the cell cytosol. These Ca^{2+} sources are utilized for signaling when exocrine cells are stimulated with a variety of hormones and neurotransmitters. It is only a fraction of the Ca^{2+} stored in the ER that is released into the cytoplasm during each Ca^{2+} spike (Petersen and Tepikin 2008), but it is sufficient to activate Ca^{2+} influx at the PM, at least at Orai1 and stromal interaction molecule 1 (STIM1) clustering domains (see below). Ca^{2+} release from the ER is mediated by Ca^{2+} channels called inositol 1,4,5-trisphosphate receptors (IP_3 receptors or IP_3Rs) (Mikoshiba 2015). Ca^{2+} is also released from lysosomes and endosomes to increase the Ca^{2+} concentration in the immediate vicinity of these organelles and regulate their activity. TRPML1 is a primary Ca^{2+} release channel in lysosomes (Di Paola et al. 2018), while TRPML3 releases Ca^{2+} from early and late endosomes (Yamaguchi et al. 2011; Grimm et al. 2014). Ca^{2+} release from lysosomes and endosomes also depends on the two-pore channels (TPCs), TPC1 (mainly endosomes) and TPC2 (mainly lysosomes) (Grimm et al. 2017a, b), because their deletion or inhibition eliminates endosomal and lysosomal Ca^{2+} release. The TPC channels are activated by phosphatidylinositol 3,5-bisphosphate ($PI(3,5)P_2$) (Xu and Ren 2015) and nicotinic acid adenine dinucleotide phosphate (NAADP) (Jha et al. 2014; Grimm et al. 2017b) to mediate the NAADP-triggered Ca^{2+} release. Ca^{2+} is also released from secretory granules and almost all known Ca^{2+} release channels were found in secretory granules, including IP_3Rs, ryanodine receptors (RyRs), and the TPCs (Petersen 2015). It is not clear whether these channels are expressed in different secretory granule populations, or whether all channels are expressed in the same granules. The major source of Ca^{2+} entry in the cytosol is provided by the PM Ca^{2+} influx channels that are activated in response to, or shortly

after, Ca^{2+} release. These include the store-operated Orai channels (Prakriya and Lewis 2015), the receptor-stimulated TRPC (Nishida et al. 2015; Chung et al. 2017), and other TRP channels (Nishida et al. 2015), which control Ca^{2+} influx across the PM.

A characteristic feature of exocrine cells is the polarized expression of receptors and signaling proteins with their prominent concentration at the apical pole. This localization results in the generation of specific Ca^{2+} signals with respect to initiation site, propagation pattern, and ultimately a physiological response. The localized Ca^{2+} signal is shown in Figure 1 (taken from Shin et al. 2001). The same pancreatic acinar cells were stimulated consecutively with the M3 agonist carbachol and then with cholecystokinin (CCK). The receptor-evoked Ca^{2+} signal starts with generation of IP_3, which then binds to the IP_3 receptors that are expressed at a high level

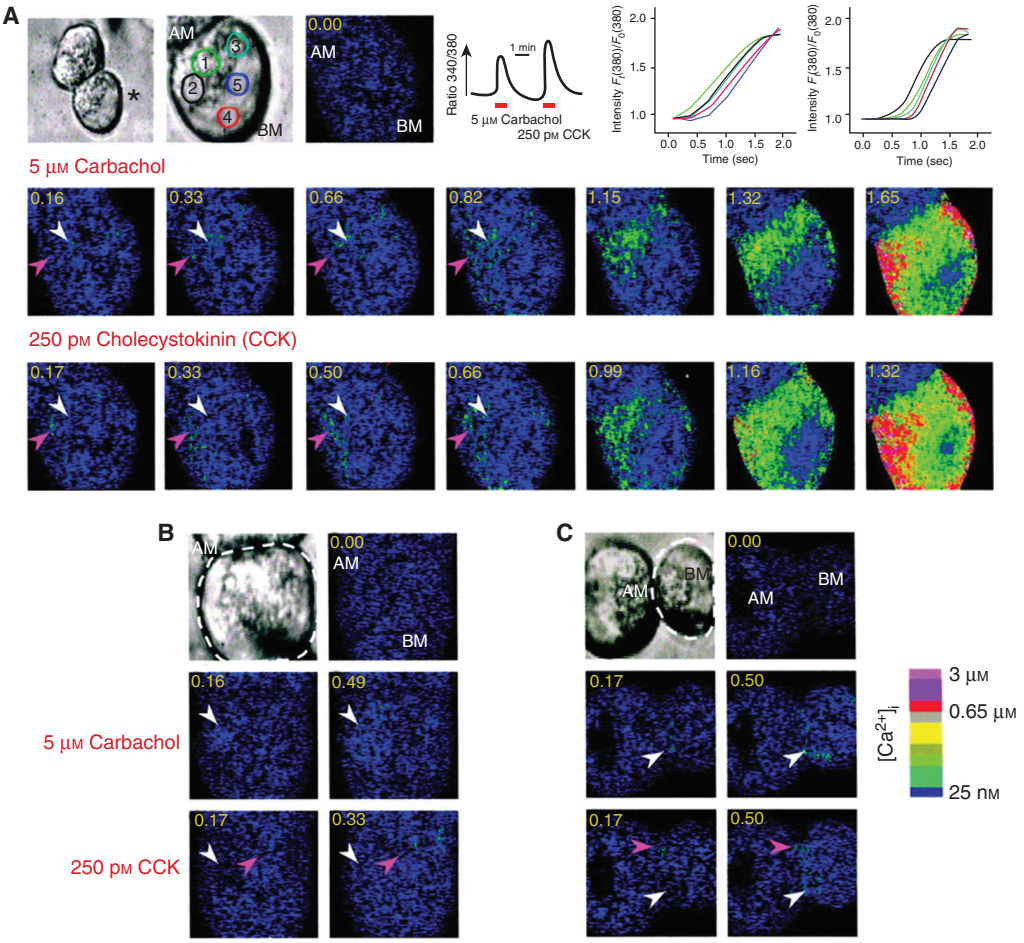

Figure 1. Receptor-specific initiation site and propagation pattern of Ca^{2+} waves. Cells were sequentially stimulated with 5 μM carbachol and 0.25 nM cholecystokinin (CCK), as indicated in the traces in the *upper* panel. (*A*) Initiation site and pattern of the Ca^{2+} wave evoked by activation of the M3 and CCK receptors in the same cell. (*B*,*C*) Additional examples of the different Ca^{2+} wave initiation sites evoked by carbachol (white arrowhead) and CCK (magenta arrowhead) stimulation in the same cells. AM, Apical membrane; BM, basal membrane. (From Shin et al. 2001; adapted, with permission, from the American Society for Biochemistry and Molecular Biology © 2001.)

at the apical pole (Lee et al. 1997b; Yule et al. 1997), thereby causing Ca^{2+} release within the apical region of the ER. Ca^{2+} released at the apical region either remains confined to the apical pole (Thorn et al. 1993; Tinel et al. 1999), or propagates in the form of a Ca^{2+} wave to the basal pole along the lateral membrane (Hong et al. 2011; Sneyd et al. 2017). Several (but not all) G-protein-coupled receptors (GPCRs) generate NAADP simultaneously with IP_3 to trigger additional Ca^{2+} release from endosomes, lysosomes, and possibly secretory granules at the apical pole (Cancela et al. 2002; Gerasimenko et al. 2006; Gerasimenko et al. 2015). Indeed, the receptors that generate NAADP, and those that do not, appear to engage different Ca^{2+} pools. This is evident from the generation of distinctive agonist-specific Ca^{2+} signals (Shin et al. 2001). Ca^{2+} release from the ER is followed by activation of PM Ca^{2+} influx channels that involves clustering of the ER Ca^{2+} sensor STIM1 (Liou et al. 2005; Roos et al. 2005) at the ER/PM junctions where it interacts with and activates Orai1 (Zhang et al. 2006; Prakriya and Lewis 2015) and TRPC channels (Bodnar et al. 2017). Interaction of STIM1-Orai1 and likely STIM1-TRPC channels take place at phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$)-rich MCS (Maléth et al. 2014).

Long-lasting elevation of cytoplasmic Ca^{2+} concentration is toxic. As a protection, cells express Ca^{2+}-regulated Ca^{2+} clearance mechanisms, including SERCA, PMCA, and SPCA, and negative feedback loops to restrict the cytosolic Ca^{2+} concentration from being continually elevated. A high cytoplasmic Ca^{2+} concentration partially inhibits the IP_3 receptors to restrict ER Ca^{2+} release (Mak and Foskett 2015), and the Orai (Prakriya and Lewis 2015; Parekh 2017) and TRPC channels (Beech 2013) to reduce Ca^{2+} influx. Some of the cytoplasmic Ca^{2+} is taken up by mitochondria to activate energy production and regulate other mitochondrial functions (Gunter et al. 2004; Petersen 2012; Giorgi et al. 2018). However, the bulk of the Ca^{2+} is reincorporated into the ER by the SERCA pumps, and part of it is extruded out of the cells by the PMCA pumps (Bruce 2018). The cycle of Ca^{2+} release, clearance, and Ca^{2+} store refilling is repeated during the period of cell stimulation, resulting in Ca^{2+} oscillations.

SIGNALING AT APICAL POLE MEMBRANE CONTACT SITES IN EXOCRINE CELLS

The cardinal functions of all epithelial cells are vectorial in nature. This is achieved by polarized cell structure with basolateral and apical membranes that are structurally and functional separated by the tight junctions. The basolateral membrane receives signals from the circulating hormones and neurotransmitters secreted by nerve endings to varicosities, whereas the apical membrane receives signals secreted by neighboring cells (hormones, proteases, and chemicals such as ATP). Signals received by receptors on one membrane (i.e., basolateral or apical) generate messengers that transmit the signals to the cell interior and to the other membrane to ensure coordination and fidelity of cellular function. This process is achieved by the signaling complexes at MCS in all cellular membranes. The ER plays a vital role in the formation of MCS as it spans the entire cytoplasm and thus establishes contacts with all cellular organelles. Moreover, signals like Ca^{2+} diffuse within the ER to deliver messages to sites where it cannot be delivered by diffusion through the cytosol because of cytoplasmic Ca^{2+} buffers (Chen et al. 2015).

The best example of signaling at MCS is Ca^{2+} signaling. Ca^{2+} signaling complexes are formed at the ER/PM junction (Muallem et al. 2017), ER/mitochondria (Tepikin 2018), ER/lysosomes (Penny et al. 2015), ER/endosomes (Kilpatrick et al. 2017), and ER/phagosomes (Nunes-Hasler and Demaurex 2017) contacts. Below, we illustrate Ca^{2+} signaling at MCS by describing signaling at the ER/PM MCS. The ER/PM junctions in secretory cells are formed mostly at the apical pole. Receptors at these junctions include several GPCRs, including the CCK, muscarinic M3, and the vasoactive intestinal peptide (VIP) receptors (Hodges et al. 1998; Shin et al. 2001). High levels of all three IP_3 receptor isoforms at the apical pole have been demonstrated by immunolocalization and functional assays (Lee et al. 1997b; Yule et al.

1997), with IP_3R2 and IP_3R3 being the dominant receptors in exocrine cells (Yule et al. 2010). The PMCA1 is localized at the lateral and basal membranes (Lee et al. 1997a), whereas the PMCA4 that respond to agonist stimulation is confined to the ER/PM junctions at the apical pole (Shin et al. 2001). Orai1 is markedly enriched at the apical pole, and the ER Ca^{2+} sensor STIM1 clusters at the apical pole upon depletion of ER Ca^{2+} (Hong et al. 2011). Exocrine cells also express the TRPC1, TRPC3, and TRPC6 channels at the apical pole (Kim et al. 2006; Hong et al. 2011). The scaffolding protein Homer1 is localized at the apical pole, binds IP_3Rs, TRPC channels, GPCRs (Worley et al. 2007; Yuan et al. 2012), and PMCA (Yang et al. 2014), and aids in the assembly of the complexes localized to the apical pole. The components of the cAMP pathway are also found at the apical pole ER/PM junctions. The membrane localization and function of Ca^{2+}-dependent adenylyl cyclases (ACs) (Nlend et al. 2007), the soluble AC (Kolodecik et al. 2012), AKAP proteins, PKA (Zinn et al. 2015), and phosphodiesterase 4D (Barnes et al. 2005) have been demonstrated in the apical pole of exocrine cells.

The assembly of Ca^{2+} signaling components at ER/PM junctions in exocrine cells results in polarized Ca^{2+} signals that are initiated at the apical pole when cells are stimulated with physiological agonist concentrations (Kasai and Augustine 1990; Thorn et al. 1993; Lee et al. 1997b; Shin et al. 2001). In this manner, an agonist-induced Ca^{2+} increase occurs at the site where exocytosis of secretory granules take place and fluid and electrolyte secretion is initiated (Lee et al. 2012). For example, granule exocytosis by pancreatic acinar cells is triggered by increases in free cytoplasmic Ca^{2+} concentration (Messenger et al. 2014). Exocytosis by salivary gland acinar cells is triggered by increases in cytoplasmic cAMP concentration (Proctor and Carpenter 2014), which is likely caused by cAMP generated at the apical pole. Fluid secretion by pancreatic, salivary, and sweat gland acinar cells follows Ca^{2+} oscillations at the apical pole.

In acinar cells, the initial increase in $[Ca^{2+}]_i$ as a result of Ca^{2+} release from the ER activates the Ca^{2+}-activated Cl^- channel anoctamin 1 (TMEM16A) at the apical membrane (Park et al. 2001; Catalán et al. 2015; Concepcion et al. 2016; Concepcion and Feske 2017) and K^+ channels at both the apical and basolateral (Romanenko et al. 2006, 2007; Almassy et al. 2012) membranes. However, ER Ca^{2+} content is limited and requires continuous replenishment by Orai1-mediated Ca^{2+} influx, without which anoctamin 1 (ANO1) activation and secretion stops, both in mouse models and in human with nonfunctional mutations, or deletion, of Orai1 (Concepcion and Feske 2017). Ca^{2+}-dependent activation of ANO1 and K^+ channels results in Cl^- and K^+ efflux and cell shrinkage. Electrical neutrality is maintained by paracellular Na^+ flow through the tight junctions. NaCl secretion dives fluid secretion through the water channel AQP5 (Zeng et al. 2017). During a Ca^{2+} spike, cell shrinkage inhibits Ca^{2+} signaling by an unknown mechanism, causing reduction in $[Ca^{2+}]_i$ that leads to reduced ANO1 and K^+ channel activity, and consequently less fluid secretion. Cell shrinkage activates the basolateral $Na^+/K^+/2Cl^-$ cotransporter NKCC1 (Melvin et al. 2005; Lee et al. 2012) to replenish K^+ and Cl^- and cell volume. The Na^+ entering the cells through NKCC1 is exchanged with K^+ by the Na^+ pump. A second Ca^{2+} spike is now initiated to trigger another fluid and electrolyte secretory cycle. Hence, acinar cells function as fluid and electrolyte secretory pumps driven by repetitive Ca^{2+} spikes (also termed Ca^{2+} oscillations).

As indicated above, all components of the cAMP signaling pathway are also localized at the apical pole ER/PM junctions in exocrine cells. Although measurement of the dynamics of receptor-stimulated cAMP generation was not reported as yet in exocrine cells, the localization of the cAMP signaling pathway at the apical pole and the cross talk and synergism between the Ca^{2+} and cAMP pathways suggest that cAMP is generated at the apical pole next to the Ca^{2+} signal. The best understanding of localized cAMP signaling in mammalian cells is in striated muscle cells. All components of the cAMP pathway are expressed at the sarcoplasmic reticulum (SR)/PM junctions (Gorelik et al. 2013; Filadi and Pozzan 2015). Receptor stimulation increases cAMP at the SR/PM junction

and activation of PKA (Rudolf et al. 2006; Filadi and Pozzan 2015). cAMP is also generated at the mitochondria surface and matrix (Lefkimmiatis et al. 2013; Filadi and Pozzan 2015). cAMP increase at the mitochondrial surface and cytoplasm were similar, but cAMP at the mitochondrial surface lasted longer and activated PKA more robustly (Lefkimmiatis et al. 2013; Burdyga et al. 2018). Matrix generation of cAMP is mediated by the soluble AC and regulates ATP synthesis (Di Benedetto et al. 2013) and aldosterone secretion in glomerulosa cells (Katona et al. 2015).

SIGNALING CROSS TALK AND SYNERGISM

The localization of the Ca^{2+} and cAMP signaling pathways at the same MCS allows for their functional interaction. Cross talk between the Ca^{2+} and cAMP occurs on many levels. cAMP/PKA phosphorylates the IP_3Rs to increase their affinity to IP_3 (Straub et al. 2004; Betzenhauser et al. 2009). PKA phosphorylates and activates PMCA (Baggaley et al. 2007) and regulates the activity of TRPC channels (Shen et al. 2011; Sung et al. 2011). PKA-mediated phosphorylation of the STIM-Orai-activating-region (SOAR) domain of STIM1 specifies the activation of the arachidonic acid-regulated Ca^{2+} selective channel rather than Orai1 (Thompson et al. 2018). Regulation of the cAMP pathway by Ca^{2+} is well documented through activation by Ca^{2+} of the Ca^{2+}-dependent ACs by Ca^{2+}. AC8, and perhaps other Ca^{2+}-dependent ACs, are activated specifically by Ca^{2+} entering through the Orai1 (Willoughby et al. 2012) and TRPC1 channels (Willoughby et al. 2014). The regulation requires the proximity of all components in the same ER/PM junctions and is dependent on the direct interaction of AC8 with Orai1 (Willoughby et al. 2012).

A prominent interaction between the Ca^{2+} and cAMP signaling pathways is the synergy between the pathways in activation of many cellular functions. In fact, most cellular functions are activated by synergism between signaling pathways to avoid overactivation that often leads to pathology (Ahuja et al. 2014). A molecular mechanism of synergy between Ca^{2+} and cAMP signaling involves PKA modulation of an IP_3R-binding protein released with inositol 1,4,5-trisphosphate (IRBIT), which interacts with the IP_3 receptors and other target proteins (shown in Fig. 2). IRBIT was identified as an IP_3 receptor-binding protein released with IP_3 (thus the name IRBIT), which interacts with the IP_3-binding site of the IP_3Rs to inhibit their activity (Ando et al. 2006). Subsequent studies have shown that IRBIT activates Na^+-HCO_3^- cotransporter (NBCe1-B), the Cl^- and HCO_3^- channel (CFTR), and the $Cl^-/2HCO_3^-$ exchanger (Slc26a6) (Shirakabe et al. 2006; Yang et al. 2009; Park et al. 2013). The function of these transporters is regulated by the With No Lysine (WNK) (Huang et al. 2007), STE20/SPS1-related proline-alanine-rich protein (SPAK) (Gagnon and Delpire 2012) kinases and the PP1 and CaMKII phosphatases (Yang et al. 2011; Vachel et al. 2018). In the resting state, IRBIT interacts with the unphosphorylated IP_3Rs and inhibits Ca^{2+} release. Upon cell stimulation with physiological concentrations of agonists that trigger cAMP production and Ca^{2+} mobilization, PKA phosphorylates the IP_3Rs, which increases their affinity for IP_3, while reducing its affinity for IRBIT, thus releasing IRBIT from the IP_3Rs (Park et al. 2013). The released IRBIT interacts with the Cl^- and HCO_3^- transporters to increase their surface expression, modulate their inhibition by intracellular Cl^-, and activates their transport function (Yang et al. 2009; Park et al. 2013; Shcheynikov et al. 2015).

TETHERING MCS

Signaling and synergism requires the formation and stabilization of MCS. This is achieved by tether proteins that facilitate the interaction of the ER with various cellular membranes. The tether proteins were initially described in yeast studies of lipid transfer between membranes and organelles. The ER/PM junctions are tethered by three tricalbins, which are anchored in the ER and interact with $PI(4,5)P_2$ in the PM (Toulmay and Prinz 2012). Another tether protein found in yeast is Ist2, which has transmembrane domains in the ER and a polybasic domain that interacts with PM $PI(4,5)P_2$ (Maass et al. 2009;

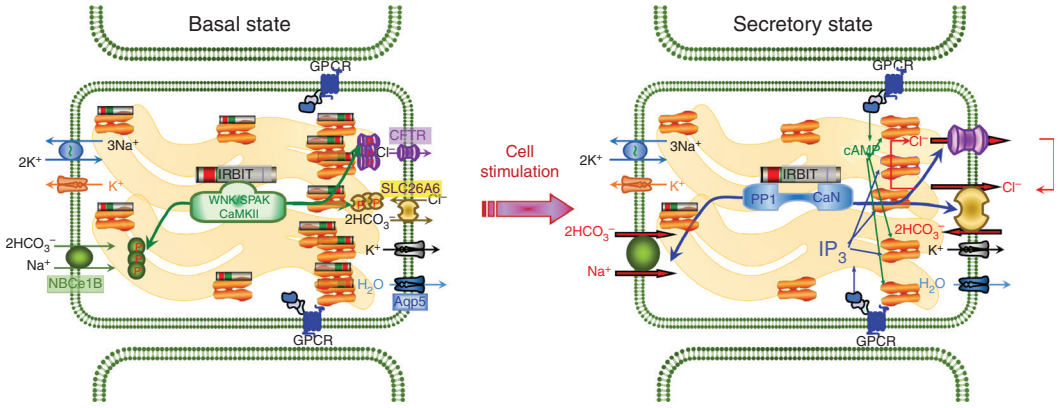

Figure 2. Molecular mechanism of synergism in cyclic AMP (cAMP) and Ca^{2+} signaling in epithelial fluid and HCO_3^- secretion. In the resting state, IRBIT recruits the WNK/SPAK and CaMKII kinases to the transporters to phosphorylate NBCe1-B autoinhibitory domain, Slc26a6 and CFTR to sequester most of them in intracellular compartments. Al low cytoplasmic IP_3, most IRBIT is bound to the IP_3 receptors, which in secretory glands are expressed at high levels at the apical pole. When the cells are stimulated with a combination of physiological concentrations of IP_3 and cAMP-generating agonists, PKA phosphorylates the IP_3Rs to facilitate the IP_3-mediated release of IRBIT from the IP_3Rs. IRBIT recruits protein phosphatase 1 (PP1) and calcineurin (CaN) to the transporters to dephosphorylate them and fuse the intracellular transporters pool with the PM. IRBIT remains bound to the transporters to relieve their constitutive inhibition and expose their intracellular Cl^- regulatory sites. The IRBIT-activated transporters mediate fluid and electrolyte secretion by CFTR-expressing epithelia.

Wolf et al. 2012). In addition, lipid transfer proteins localize to the ER/PM junctions, some of which do not have ER-interacting domains. Instead, these proteins have two phenylalanine in acidic tract motifs that interact with the ER-localized vesicle-associated membrane protein-associated proteins (VAPs) (Stefan et al. 2011).

The mammalian homologs of the tricalbins are the three extended synaptotagmins (E-Syts) (Min et al. 2007; Giordano et al. 2013). The E-Syts are anchored in the ER and interact with PM $PI(4,5)P_2$ through their C2 domains (Giordano et al. 2013). Although all the E-Syts tether the ER to the PM (Giordano et al. 2013), they have distinct functions. E-Syt1, but not E-Syt2 and E-Syt3, affect STIM1-Orai1 function at the ER/PM junctions (Maléth et al. 2014; Cao et al. 2015), while E-Syt2 and E-Syt3, but not E-Syt1, mediate receptor endocytosis (Jean et al. 2010) and E-Syt2, but not E-Syt1 and E-Syt3, recruits phosphatidylinositol 4-phosphate (PI4P) phosphatase to the ER/PM junctions to control $PI(4,5)P_2$ levels (Dickson et al. 2016). Sequence similarity suggests that the mammalian homolog of Ist2 is a member of the 10 anoctamins family (ANOs, also known as TMEM16A-J) (Caputo et al. 2008; Schroeder et al. 2008; Yang et al. 2008). The mammalian VAPs bind the lipid exchange Nir proteins that maintain the PM level of $PI(4,5)P_2$ by exchanging phosphatidic acid with $PI(4,5)P_2$ (Kim et al. 2013, 2015). Other important proteins in the ER/PM junctions are ORP5 and ORP8, which play a role in lipid transfer and control the PM level of phosphatidylserine (PS) by exchanging PM PI4P with ER synthesized PS (Chung et al. 2015). ORP5 and ORP8 have also been found at the ER-mitochondria MCS and are proposed to have a role in mitochondrial function (Galmes et al. 2016).

The ER Ca^{2+} sensors, STIM1 and STIM2 (hereafter denoted STIMs), are clustered at the ER/PM junctions in response to Ca^{2+} release from the ER, after which they have key roles in ER/PM junction stabilization. The STIMs have a single transmembrane domain that spans the ER membrane and a carboxy-terminal polybasic domain, which, in response to ER Ca^{2+} depletion, binds to PM $PI(4,5)P_2$ and to Orai1 (Liou et al. 2005; Roos et al. 2005; Zhang et al. 2006;

Brandman et al. 2007). The STIMs have a Ca^{2+}-binding EF-hand and an SAM domain that reside in the ER lumen. When Ca^{2+} is bound to the EF-hand, the STIMs do not tether the ER and PM. The cytoplasmic portion of the STIMs starts with three coiled-coil domains that regulate unfolding of STIM1 (Korzeniowski et al. 2010; Muik et al. 2011) to release the SOAR domain (Yuan et al. 2009), also known as CAD (Park et al. 2009) and CCb9 (Kawasaki et al. 2009). The STIM1 SOAR domain interacts with the carboxy- and amino-termini of Orai1 to activate the channel (Park et al. 2009; Yuan et al. 2009). SOAR is followed by the carboxyl-terminus inhibitory domain (CTID) (Jha et al. 2013), serine/proline rich, and polybasic domains (Liou et al. 2005; Roos et al. 2005). STIM2 is a poor activator of Orai1 (relative to STIM1) but affects Ca^{2+} signaling by functioning as a tether to enhance the efficiency of receptor-stimulated Ca^{2+} signaling (Ong et al. 2015) by facilitating STIM1 conformational change required for STIM1 interaction with and activation of Orai1 (Subedi et al. 2018).

Ca^{2+} SIGNALING AT THE ER/PM JUNCTIONS IN PATHOLOGY

Excessive or prolonged increases in cytoplasmic Ca^{2+} concentration occurs in many pathological states, leading to cell death by disrupting several cellular processes, including mitochondrial function (Pizzo et al. 2012; Dorn and Kitsis 2015), apoptosis (Dong et al. 2006), gene regulation (Parekh 2010), and membrane transport (Lee et al. 2012; Maléth and Hegyi 2014). Uncontrolled Ca^{2+} influx is the result of sustained activation of the Ca^{2+} influx channels, which can be caused either by excessive Ca^{2+} release from the ER, or lack of Ca^{2+} influx inactivation. In exocrine cells TRPC1, TRPC3, and Orai1 are the Ca^{2+} influx channels activated by GPCR stimulation (Choi et al. 2014; Cao et al. 2015). Excessive epithelial Ca^{2+} influx causes several pathologies (Hecquet and Malik 2009; Eisenhut and Wallace 2011; Gerasimenko et al. 2014; Ilatovskaya and Staruschenko 2015).

Acute pancreatitis is a life-threatening disease with multiple etiologies and no cure, and is caused by ductal obstruction, bile acid reflux, or excessive alcohol consumption. Sustained Ca^{2+} increase, because of excessive Ca^{2+} influx, is the nodal point in triggering the various forms of acute pancreatitis (Gerasimenko et al. 2014; Maléth and Hegyi 2014). Indeed, in animal models, genetic or pharmacological inhibition of Ca^{2+} influx channels can prevent, and even reverse, the disease after its initiation. However, unfortunately, inhibition of the two principal Ca^{2+} influx channels, TRPC and Orai1, has significant drawbacks. The advantage of inhibiting TRPC channels is that minor phenotypes were observed in the deletion of TRPC1 (Liu et al. 2007), TRPC3 (Kim et al. 2009), TRPC6 (Dietrich et al. 2005), and a combination of these channels in mice (Nilius and Flockerzi 2014; Sexton et al. 2016). Indeed, knockout of TRPC3 or inhibition of TRPC3 with Pyrazol 3 in mice markedly reduced the tissue damage caused by induction of acute pancreatitis (Kim et al. 2009, 2011). Unfortunately, inhibition of TRPC3 protected against acute pancreatitis only if the channel was inhibited prior to induction of the disease and was not effective in reversing the damage when applied after initiation of the disease (Kim et al. 2009, 2011).

Similarly, inhibitors of Orai1 prevented the tissue damage due to acute pancreatitis in both in vitro assays and in mice (Gerasimenko et al. 2013; Wen et al. 2015). Pharmacological inhibition of Orai1 partially protected against all models of acute pancreatitis, induced by excessive agonist stimulation, infusion of bile acids or of alcohol metabolites, even after initiation of the disease (Wen et al. 2015). Another significant advantage of Orai1 inhibitors is that they will likely inhibit the inflammation associated with all forms of acute pancreatitis (Sah et al. 2013) as Orai1-mediated Ca^{2+} influx is critical for activation of various inflammatory cells (Shaw and Feske 2012). However, one serious problem with the use of Orai1 inhibitors is that Ca^{2+} influx mediated by Orai1 is essential for many cellular functions in all cell types. Thus, inhibitors of Orai1 are expected to have serious adverse side effects. For example, in the pancreas, targeted induced deletion of Orai1 only in acinar cells resulted in 70% mortality because of

bacteremia and sepsis (Ahuja et al. 2017). Pancreatic acinar cells synthesize and secrete large quantities of antibacterial agents to the gut to control the gut microbiota. Deletion of acinar cells' Orai1 inhibited synthesis and secretion of the antibacterial agents to the gut, resulting in dysbiosis that proved to be lethal (Ahuja et al. 2017). Hence, caution must be used in the application of Orai1 inhibitors to treat diseases resulting from excessive Ca^{2+} influx. One possible approach is to use a combination of TRPC channel blockers and low-dose Orai1 inhibitors to treat diseases like acute pancreatitis. Another is to use small molecules to control the function of the Ca^{2+} influx regulators store-operated calcium entry (SOCE)-associated regulatory factor (SARAF) (Jha et al. 2013) and the STIM-activating enhancer (STIMATE) (Jing et al. 2015; Quintana et al. 2015) that controls the function of STIM1.

CONCLUDING REMARKS

Aberrant Ca^{2+} signals have been known to be the root cause of many cell damage– and inflammation-associated diseases. Other, less appreciated, aspects of signaling associated with pathology is aberrant synergy between signaling pathways, in particular, cross talk and synergy between the Ca^{2+} and cAMP/PKA pathways. Recent evidence highlights the importance of organizing signaling complexes at MCS to mediate the synergy and the role of tether proteins in the formation of MCS. Therefore, a more complete understanding of the formation of MCS and their role in the organization of cell signaling and in cross talk and synergism is essential for understanding basic aspects of cell regulation and, importantly, the alterations that occur in disease states. This should allow development of a more precise and perhaps better targeted intervention to treat aberrant cell signaling in disease states.

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