Ca\(^{2+}\) tunnelling through the ER lumen as a mechanism for delivering Ca\(^{2+}\) entering via store-operated Ca\(^{2+}\) channels to specific target sites

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Abstract  Ca\(^{2+}\) signalling is perhaps the most universal and versatile mechanism regulating a wide range of cellular processes. Because of the many different calcium-binding proteins distributed throughout cells, signalling precision requires localized rises in the cytosolic Ca\(^{2+}\) concentration. In electrically non-excitable cells, for example epithelial cells, this is achieved by primary release of...
Ca\(^{2+}\) from the endoplasmic reticulum via Ca\(^{2+}\) release channels placed close to the physiological target. Because any rise in the cytosolic Ca\(^{2+}\) concentration activates Ca\(^{2+}\) extrusion, and in order for cells not to run out of Ca\(^{2+}\), there is a need for compensatory Ca\(^{2+}\) uptake from the extracellular fluid. This Ca\(^{2+}\) uptake occurs through a process known as store-operated Ca\(^{2+}\) entry. Ideally Ca\(^{2+}\) entering the cell should not diffuse to the target site through the cytosol, as this would potentially activate undesirable processes. Ca\(^{2+}\) tunnelling through the lumen of the endoplasmic reticulum is a mechanism for delivering Ca\(^{2+}\) entering via store-operated Ca\(^{2+}\) channels to specific target sites, and this process has been described in considerable detail in pancreatic acinar cells and oocytes. Here we review the most important evidence and present a generalized concept.

Introduction

Effective and precise intracellular Ca\(^{2+}\) signalling depends on specific Ca\(^{2+}\) sensors and transport proteins expressed differentially on organelle and plasma membranes, as well as Ca\(^{2+}\) buffers with different affinities and kinetics in different cellular compartments (Petersen et al. 1994; Berridge, 2016). Because Ca\(^{2+}\) can interact with many potential cellular targets, signalling precision requires localized rises of the cytosolic \([\text{Ca}^{2+}]_i\) (\([\text{Ca}^{2+}]_i\)) (Petersen et al. 1994; Petersen & Verkhratsky, 2016). In the nervous system, the extremely precise control of presynaptic neurotransmitter secretion depends on close co-localization of voltage-gated Ca\(^{2+}\) channels and the exocytotic machinery (Südhof, 2013). However, the target for the action of Ca\(^{2+}\) cannot always be very close to the site of Ca\(^{2+}\) entry. A prime example of such a scenario comes from the physiology of the pancreatic acinar cells, where Ca\(^{2+}\) entry occurs at the base of the cell, whereas the control of secretion has to take place at the opposite end of the cell at the apical pole (Petersen et al. 1994; Petersen & Tepikin, 2008).

In these and many other electrically non-excitable cells, which do not have voltage-gated Ca\(^{2+}\) channels and do not fire action potentials (Petersen, 1992), the primary Ca\(^{2+}\) movement is from the endoplasmic reticulum (ER) into the cytosol (Nielsen & Petersen, 1972; Berridge, 2016) and this, in turn, triggers store-operated Ca\(^{2+}\) entry (Putney, 1986; Petersen & Tepikin, 2008; Parekh, 2010). Diffusion of Ca\(^{2+}\) through the cytosol, from an entry site to a distant target, would potentially activate many inappropriate processes and a mechanism that could avoid this path would therefore be advantageous. Transport through an organelle, moving Ca\(^{2+}\) from its entry point to its target, would solve this problem (Fig. 1). The process of Ca\(^{2+}\) tunnelling through the ER was discovered in studies on pancreatic acinar cells carried out 20 years ago (Mogami et al. 1997). A similar process was later described in dopamine neurons (Choi et al. 2006) and the whole concept has more recently been generalized, based on experiments in oocytes (Courjaret & Machaca, 2014; Courjaret et al. 2016a). Furthermore, in a recent study (Kar et al. 2016), it has been shown that Ca\(^{2+}\) refilling of the
nuclear envelope, after inositol trisphosphate (IP$_3$)-evoked Ca$^{2+}$ release into the nucleoplasm through the inner nuclear membrane (Gerasimenko et al. 1995), depends on Ca$^{2+}$ (entering via store-operated Ca$^{2+}$ channels) being tunnelled through the ER lumen directly into the nuclear envelope. In this article, we describe and review the most important evidence for Ca$^{2+}$ tunnelling, primarily based on studies of pancreatic acinar cells and oocytes.

**Spatial and temporal features of Ca$^{2+}$ signals**

Specificity in Ca$^{2+}$ signals is encoded in their spatial, temporal and amplitude features. These Ca$^{2+}$ dynamics combine to activate a defined subset of Ca$^{2+}$-dependent downstream effectors to transduce the cellular response (Berridge et al. 2000, 2003). Spatially, Ca$^{2+}$ signals are tightly regulated and are typically initiated by elementary events due to the opening of Ca$^{2+}$ channels (intracellular or at the cell membrane). These elementary events due to the opening of one or a few channels can either remain localized resulting in Ca$^{2+}$ signals in the microdomain around the channel(s), or coalesce through complex mechanisms into more global Ca$^{2+}$ events that often encompass the entire cell (Berridge, 1997).

The extent and speed of Ca$^{2+}$ movement is heavily influenced by the concentration and characteristics of the available Ca$^{2+}$ buffers. It was shown many years ago, that adding a low affinity mobile Ca$^{2+}$ buffer to the cytosol can profoundly change the timing and spatial extension of agonist-elicited cytosolic Ca$^{2+}$ signals (Petersen et al. 1991). The cytosolic Ca$^{2+}$ buffering characteristics vary markedly between different cell types with, for example, a high level of relatively low mobility buffer in pancreatic acinar cells (Mogami et al. 1999), and a less restricted environment for Ca$^{2+}$ diffusion in oocytes (Allbritton et al. 1992) and some nerve cells (Lin et al. 2017). Inevitably, [Ca$^{2+}$]$_i$ measurements using Ca$^{2+}$-sensitive fluorescent probes will be influenced by the Ca$^{2+}$-binding properties of the probes, so unless a careful analysis of the Ca$^{2+}$ buffering situation has been carried out, as recently described by Lin et al. (2017), some caution with regard to interpreting quantitative results is called for.

In general, the diffusion of Ca$^{2+}$ in the cytosol is always severely limited, as compared to movement in water, due to the relatively high buffering capacity. Some estimates indicate that free Ca$^{2+}$ diffuses < 0.1 μm, and is free for ~0.5 μs before it is buffered (Allbritton et al. 1992; Kasai & Petersen, 1994). At the mouth of an open Ca$^{2+}$ channel and given the great concentration gradients across both the ER and plasma membrane, Ca$^{2+}$ flow rapidly overwhelms the local buffering capacity resulting in a microdomain of high Ca$^{2+}$ concentration in the order of 20–200 μM (Rizzuto & Pozzan, 2006). The spatial spread of these high Ca$^{2+}$ microdomains is thus very tightly controlled, and is predicted based on theoretical modelling to be maintained within 20 nm of the channel (Simon & Llinas, 1985; Neher, 1998). Beyond the immediate point source of Ca$^{2+}$ entry at the mouth of the channel, Ca$^{2+}$ diffusion creates a downward gradient away from the channels that is thought to dissipate to the submicromolar range within 200 nm of the channel (Neher, 1998; Shuai & Parker, 2005; Demuro & Parker, 2006). This provides for an elegant mechanism to activate Ca$^{2+}$-dependent effectors that localize within the spatial spread of Ca$^{2+}$ signals generated due to elementary Ca$^{2+}$ events (Rizzuto & Pozzan, 2006; Parekh, 2008).

Global Ca$^{2+}$ signals, in contrast, have a significantly broader spread on the order of 10–100 μm. This highlights a spatial gap between elementary and global Ca$^{2+}$ signals, as there could be a physiological need to activate effectors that are not in the immediate vicinity of a Ca$^{2+}$ channel without inducing a global Ca$^{2+}$ rise that would activate a multitude of other signalling pathways. Although not discussed in details here, cells could maintain specificity in their Ca$^{2+}$ signals despite the extent of their spatial spread by controlling their amplitude and frequency for example. Nonetheless, there are several examples that argue that Ca$^{2+}$ signals activate effectors in the...
Ca\(^{2+}\) signalling in electrically non-excitatory cells is typically initiated downstream of agonist stimulation through the activation of a phospholipase C that hydrolyses phosphatidylinositol biphosphate (PIP\(_2\)) at the plasma membrane and results in the production of IP\(_3\) and diacylglycerol. IP\(_3\) diffuses and gates open IP\(_3\) receptors at the ER membrane, releasing store Ca\(^{2+}\) to mediate the first phase of the Ca\(^{2+}\) signal. Should the Ca\(^{2+}\) release phase result in significant store depletion, it leads to the activation of Ca\(^{2+}\)-influx at the plasma membrane through store-operated Ca\(^{2+}\) entry (SOCE). SOCE is mediated by members of the stromal interaction molecule (STIM) and Orai family (Prakriya & Lewis, 2015). STIM1 is a single-pass ER membrane protein with luminal EF hands allowing it to sense the ER Ca\(^{2+}\) concentration (Liou et al., 2005; Roos et al., 2005). Store depletion results in STIM1 losing its luminal bound Ca\(^{2+}\) leading to a conformational change in the protein and its clustering and translocation to ER–plasma membrane (PM) junctions that are 20 nm apart (Luik et al., 2006; Prakriya et al., 2006; Statopoulos et al., 2006; Vig et al., 2006; Wu et al., 2006; Yeromin et al., 2006; Liou et al., 2007). The close proximity of the ER and PM at these junctions allows STIM1 to span the distance and physically interact with Orai1 at the PM. Orai1 is a four transmembrane domain protein that forms a hexameric channel that is exclusively Ca\(^{2+}\) selective (Hou et al., 2012). STIM1 clusters stabilize at ER–PM junctions initially through interaction of the poly-lysine domain at the C-terminal end of STIM1 with PIP\(_2\) in the PM. Activated STIM1 in response to store depletion exposes the STIM1 Orai1-activating region (SOAR)/CRAC-activating domain, which interacts with Orai1, traps it within the STIM1-defined ER–PM junctions, and gates it open, thus allowing Ca\(^{2+}\) flow into the cell. SOCE activation not only results in store refilling but also shapes Ca\(^{2+}\) signal dynamics. There is therefore a tight functional link between IP\(_3\)-dependent Ca\(^{2+}\) release in response to agonist stimulation and Ca\(^{2+}\) influx through SOCE.

### Ca\(^{2+}\) tunnelling in pancreatic acinar cells

**The function of the acinar cells.** The principal function of the exocrine pancreas is to deliver digestive enzymes to the intestine in order to break down food products, so that they can be absorbed into the circulation. The most important secretory cell in the exocrine pancreas is the acinar cell, which manufactures the inactive pro-enzymes and stores them in zymogen granules. When enzyme delivery is required, the acinar cells receive a signal in the form of either the neurotransmitter acetylcholine (ACh; released from parasympathetic nerve endings) and/or the hormone cholecystokinin (CCK). Interaction with specific surface membrane receptors activates signal transduction mechanisms that generate intracellular Ca\(^{2+}\), liberating messengers – IP\(_3\) in the case of ACh and cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate (NAADP) in the case of CCK stimulation – thereby releasing Ca\(^{2+}\) from intracellular stores (Petersen & Tepikin, 2008). As a consequence of the depletion of intracellular Ca\(^{2+}\) stores, Ca\(^{2+}\)-permeable channels in the plasma membrane are opened allowing Ca\(^{2+}\) entry from the extracellular solution (Petersen & Tepikin, 2008).

Under physiological conditions the cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) changes evoked by ACh or CCK consist of repetitive short-lasting elevations mostly confined to the apical region (Kasai et al., 1993; Thorn et al., 1993), where the zymogen granules are concentrated. This local rise in [Ca\(^{2+}\)]\(_i\) triggers exocytosis of the granule content (Maruyama & Petersen, 1994) as well as opening up Cl\(^-\) channels in the apical membrane and K\(^+\) channels in the apical part of the lateral membrane (Petersen & Maruyama, 1984; Petersen, 1992; Park et al., 2001b). These channel openings enable operation of the Na\(^+\)/K\(^+\)/2Cl\(^-\) co- transporter as well as increased turnover of the Na\(^+\)/K\(^+\) pump across the basolateral membrane (Petersen, 1992). The net result is uptake of Cl\(^-\) across the basolateral membrane and secretion of Cl\(^-\) across the apical membrane with Na\(^+\) following via a paracellular pathway through the leaky tight junctions. Water moves along with the salt both through the cell and through the so-called tight junctions (Petersen, 1992). The Ca\(^{2+}\)-activated acinar fluid secretion is a vehicle for transport of the pro-enzymes into the duct system, where an additional ductal fluid secretion of a secretin-stimulated bicarbonate-rich solution (Scratcherd et al., 1981; Lee et al., 2012) will help wash the pro-enzymes into the gut where they become active digestive enzymes.

**The polarity of the acinar cells.** The acinar cells secrete enzymes and fluid in one direction, namely into the lumen of the acinar unit, which is directly connected to the duct
system and, therefore, these cells are highly polarized. The
zymogen granules are in the apical part of the cells, whereas
the nucleus – surrounded by densely packed rough ER – occu-
pies the basolateral region. The apical membrane area is
much smaller than the basolateral membrane area, but the
final stage of secretion occurs exclusively through the
apical membrane. The tight junctions, which are leaky
in the case of the acinar epithelium, are placed close to
the acinar lumen. Although the bulk of the ER is in the
basolateral region, ER elements penetrate into the apical
zymogen granule-rich region all the way to the apical
membrane (Gerasimenko et al. 2002).

With regard to the localization of the principal
Ca\(^{2+}\)-activated ion channels in the plasma membrane of
the acinar cells, we know that the Ca\(^{2+}\)-activated Cl\(^{-}\)
channels, transmembrane member 16A (TMEM16A)/
anoctamin-1 (ANO1) (Lee et al. 2012), are exclusively
present in the apical membrane (Park et al. 2001b),
whereas the high-conductance and voltage-sensitive
Ca\(^{2+}\)-activated K\(^{+}\) channels (present in the pig and
human acinar cells) are found in the basolateral
membrane (Maruyama et al. 1983; Petersen et al. 1985).
Simultaneous patch clamp recording of Cl\(^{-}\) conductance
and capacitance indicate that during normal physiological
stimulus–secretion coupling, each local apical Ca\(^{2+}\) spike
causes near-synchronous (but see below) opening of Cl\(^{-}\)
channels and exocytosis (Maruyama & Petersen, 1994).

With regard to Ca\(^{2+}\) transport across the plasma
membrane, we know that Ca\(^{2+}\)-ATPase-driven Ca\(^{2+}\)
extrusion occurs mostly through the apical membrane
(Belan et al. 1996), whereas store-operated Ca\(^{2+}\) entry
occurs through the basolateral membrane (Mogami et al.
1997; Park et al. 2001a; Lur et al. 2009).

How Ca\(^{2+}\) entering through the basolateral plasma
membrane allows Ca\(^{2+}\) signal generation near the
apical membrane without passing through the cytosol.
Although it has been known from the earliest days of
work on stimulus–secretion coupling in pancreatic acinar
cells that the initial Ca\(^{2+}\) signal generation evoked by
stimulation with either ACh or CCK is due to release of
Ca\(^{2+}\) from internal stores (Matthews et al. 1973; Petersen
& Ueda, 1976), it has also been clear that supply of Ca\(^{2+}\)
from the extracellular solution is essential for continuation
of secretion (Petersen & Ueda, 1976). The reason for the
extracellular Ca\(^{2+}\) requirement is that every rise in the
cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)])i inevitably activates
Ca\(^{2+}\) pumps in the plasma membrane (plasma membrane
Ca\(^{2+}\)-activated ATPase; PMCA) resulting in extrusion
of Ca\(^{2+}\) (Tepkin et al. 1992), which then has to be
compensated by Ca\(^{2+}\) entry, as otherwise the cell would
gradually run out of Ca\(^{2+}\).

Agonist-elicited cytosolic Ca\(^{2+}\) spiking, which is the
normal physiological signal for secretion, requires a
relatively high [Ca\(^{2+}\)]i in the lumen of the ER (Park et al.
2000). In experiments on isolated acinar cells where [Ca\(^{2+}\)]i
changes in both the cytosol and the lumen of the ER
were measured simultaneously, it could be shown that
ACh evokes cytosolic Ca\(^{2+}\) spiking for several minutes
in the absence of external Ca\(^{2+}\). However, spiking sub-
sequently stops after only a relatively modest reduction
of [Ca\(^{2+}\)]ER (Park et al. 2000). Thus, Ca\(^{2+}\) entry through
store-operated Ca\(^{2+}\) channels, refilling the ER store, is
essential for the normal physiological function of the
acinar cells.

The original concept of Ca\(^{2+}\) tunnelling through the
ER lumen, from entry at the base of the cell to release
near the apical membrane (Fig. 1), was based on
experiments in which isolated acinar cells were kept
in a Ca\(^{2+}\)-free solution with a patch pipette attached
to the basal surface (Mogami et al. 1997). The patch
pipette was filled with a Ca\(^{2+}\)-containing solution and
Ca\(^{2+}\) entry across the membrane covered by the pipette
tip could be regulated by controlling the pipette potential.
After supra-maximal ACh stimulation had emptied
the intracellular stores during a period without Ca\(^{2+}\) entry
(negative – retaining – potential in the pipette), ACh
stimulation was discontinued and a period of Ca\(^{2+}\) entry
was enabled by switching the pipette potential from
negative to positive. No change was observed in [Ca\(^{2+}\)],
during this Ca\(^{2+}\) entry period, but after discontinuation
of Ca\(^{2+}\) entry (switching the pipette voltage back to negative),
a new period of ACh stimulation caused a local rise of
[Ca\(^{2+}\)]i in the apical pole near the apical membrane,
causally as under normal conditions. A rise in [Ca\(^{2+}\)], near
the cell-attached pipette during Ca\(^{2+}\) entry could only be
observed when the ER Ca\(^{2+}\) pumps (sarco/endoplasmic
reticulum Ca\(^{2+}\)-activated ATPase; SERCA) were arrested
by thapsigargin (Thastrup et al. 1989). However, in this
situation there was no sign of transfer of Ca\(^{2+}\) from
the base to the apex, as ACh stimulation after a period of
Ca\(^{2+}\) entry failed to elicit any Ca\(^{2+}\) release in the apical
region (Mogami et al. 1997). As thapsigargin is a very
selective blocker of SERCA pumps (Thastrup et al. 1989),
the simplest explanation for the phenomenon observed,
 namely the non-cytosolic transfer of Ca\(^{2+}\) across the cell
from base to lumen, is movement through the ER lumen.

The ER Ca\(^{2+}\) tunnelling concept (Mogami et al. 1997)
assumed that Ca\(^{2+}\) would move more easily within the
lumen of the ER than in the cytosol (Fig. 1). The relatively
low mobility of Ca\(^{2+}\) in the cytosol was demonstrated in
the classical experiments of Baker & Crawford (1972)
on axoplasm, in which it could be shown that radioactive
Mg\(^{2+}\) moved much more quickly than radioactive Ca\(^{2+}\),
and later confirmed by Allbritton et al. (1992). In the
acinar cells, based on measurements of absolute calcium
movements and changes in [Ca\(^{2+}\)]i in the cytosol and the
ER, we estimated that the calcium binding capacity in the
ER lumen is about 20 whereas in the cytosol it is about
1500–2000 (Mogami et al. 1999). Thus the mobility of
$\text{Ca}^{2+}$ in the ER lumen is very much higher than in the cytosol (Fig. 1). The high mobility of $\text{Ca}^{2+}$ in the ER lumen was demonstrated directly by experiments in which changes in $[\text{Ca}^{2+}]_{\text{ER}}$ at various locations in the ER could be monitored after a highly localized uncaging of caged $\text{Ca}^{2+}$ in the ER lumen (Park et al. 2000). These experiments showed that after a local $\text{Ca}^{2+}$ uncaging event, rises in $[\text{Ca}^{2+}]_{\text{ER}}$ were observed quickly over considerable distances (more than 10 $\mu$m away from the site of uncaging) and that the whole of the ER was re-equilibrated with regard to $[\text{Ca}^{2+}]_{\text{ER}}$ within a few seconds (less than the time interval between cytosolic $\text{Ca}^{2+}$ spikes during physiological $\text{Ca}^{2+}$ signalling) (Park et al. 2000; Petersen et al. 2001).

**Movement of $\text{Ca}^{2+}$ from the extracellular fluid into the ER lumen at the base.** Early work on perfused submandibular glands showed that ACh-evoked intracellular $\text{Ca}^{2+}$ release was followed, after a delay, by $\text{Ca}^{2+}$ influx into the gland cells from the perfusion fluid (Nielsen & Petersen, 1972). Later, work on isolated pancreatic acinar cells showed more precisely that the $\text{Ca}^{2+}$ entry, following the ACh-evoked immediate (< 0.5 s) apical $\text{Ca}^{2+}$ release, occurred through the basolateral membrane after a delay of about 6–7 s (Toescu & Petersen, 1995). A few years later it was shown that it is possible to refill the emptied ER with $\text{Ca}^{2+}$ flowing into the cell from a point source at the base of the cell (Mogami et al. 1997). Like in many other cell types, store-operated $\text{Ca}^{2+}$ entry is mediated by translocation of STIM to puncta near the plasma membrane, which in the pancreatic acinar cells are specifically located at the basolateral part of the cell (Lur et al. 2009). However, in these cells there is a specific challenge for this process, as the ER is of the rough type due to the presence of ribosomes. The size of ribosomes is such that it would not allow the close molecular interaction between STIM in the ER membrane and $\text{Ca}^{2+}$ channels in the plasma membrane that is necessary for channel activation. However, it turns out that there are small areas of the otherwise rough ER that are devoid of ribosomes, allowing these parts to come very close to the plasma membrane (Lur et al. 2009) (Fig. 2).

The biophysical nature of the $\text{Ca}^{2+}$ entry process in the pancreatic acinar cells was not clarified until recently (Gerasimenko et al. 2013), when patch clamp whole-cell current recording studies showed that the inward $\text{Ca}^{2+}$ current evoked by ER store $\text{Ca}^{2+}$ depletion has characteristics very similar to the $\text{Ca}^{2+}$ release activated $\text{Ca}^{2+}$ (CRAC) current previously discovered in immune cells (Hoth & Penner, 1992; Feske, 2007; Parekh, 2010), and could be blocked by a specific CRAC channel inhibitor (Gerasimenko et al. 2013). During $\text{Ca}^{2+}$ refilling of the ER, for example after ACh-evoked emptying of the store, there is no measurable increase in the cytosolic $\text{Ca}^{2+}$ concentration near the $\text{Ca}^{2+}$ entry channels although, as mentioned above, it is possible to observe a rise in the cytosolic $[\text{Ca}^{2+}]$ during $\text{Ca}^{2+}$ entry if the SERCA pumps in the ER have been arrested by thapsigargin (Mogami et al. 1997). In that case there is also a clear increase in the $[\text{Ca}^{2+}]$ in the mitochondria (mitochondrial $\text{Ca}^{2+}$ uptake being mediated by the mitochondrial $\text{Ca}^{2+}$ uniporter; MCU; De Stefani et al., 2011, 2016) placed very close to the basolateral membrane (Park et al. 2001a) (Fig. 2). $\text{Ca}^{2+}$ uptake into these peripheral mitochondria is functionally important as it will increase ATP production (De Stefani et al. 2016) locally, thereby fuelling the SERCA pumps. It would therefore appear that the crucial molecules, involved in the process of moving $\text{Ca}^{2+}$ from the extracellular fluid into the ER, namely CRAC channels, SERCA pumps and the MCU, are localized very close together in the basolateral part of the cell during SOCE (Fig. 2).

**Movement of $\text{Ca}^{2+}$ from the ER into the apical cytosol where activation of exocytosis and Cl$^{-}$ channels occurs.** The rise in $[\text{Ca}^{2+}]$, evoked by either ACh or CCK...
stimulation, always starts in the apical part of the cell, close to the apical membrane (Kasai & Augustine, 1990; Kasai et al. 1993; Thorn et al. 1993; Cancela et al. 2000) and, at near-physiological intensities of stimulation, the rise is mostly confined to the apical region (Kasai et al. 1993; Thorn et al. 1993), due to the perigranular mitochondrial firewall (Tinel et al. 1999; Park et al. 2001a). Even under conditions where muscarinic receptor activation occurs exclusively within a small region at the base of the cell (uncaging of caged carbachol in a cell-attached patch pipette), the rise in \([\text{Ca}^{2+}]_i\), always starts near the apical membrane (Ashby et al. 2003). Close comparison of the time course of the increases in Cl\(^-\) conductance and capacitance (indicative of fusion between zymogen granules and apical plasma membrane) during individual apical \(\text{Ca}^{2+}\) spikes shows (Fig. 3) that Cl\(^-\) channels are activated slightly earlier than the start of exocytosis and that the Cl\(^-\) conductance increase slightly outlasts the period of increased capacitance (Maruyama & Petersen, 1994). This could be explained either by the Cl\(^-\) channels being located closer to the ER \(\text{Ca}^{2+}\) release channels than the sites of exocytosis, or by the Cl\(^-\) channels being more sensitive to the local \([\text{Ca}^{2+}]_i\) changes than the exocytosis machinery.

\(\text{Ca}^{2+}\) spiking, induced by stimulation with either ACh or CCK, is abolished by blockade of IP\(_3\) receptors (IP\(_3\)Rs) (Wakui et al. 1990), but also by blockade of ryanodine receptors (RyRs) (Canceda et al. 2000). Since \(\text{Ca}^{2+}\) spiking can also be elicited by intracellular \(\text{Ca}^{2+}\) infusion (Osipchuk et al. 1990; Wakui et al. 1990), it is probably due to interactive \(\text{Ca}^{2+}\)-induced \(\text{Ca}^{2+}\) release, involving both IP\(_3\)Rs and RyRs. The mechanisms by which ACh and CCK initiate apical \(\text{Ca}^{2+}\) signal generation are different. In the case of ACh stimulation, there is IP\(_3\) generation due to phospholipase C activation whereas in the case of physiological CCK stimulation (low picomolar CCK concentration), there is primary generation of NAADP (Yamasaki et al. 2005). Thus blockade of NAADP receptors inhibits CCK- but not ACh-elicited \(\text{Ca}^{2+}\) spiking (Canceda et al. 2000; Gerasimenko et al. 2015). In spite of these mechanistic differences, the measurable \(\text{Ca}^{2+}\) signal progression from the initiation site near the apical membrane towards the perigranular mitochondrial belt is quantitatively very similar in both cases (Canceda et al. 2000). This indicates that the initial trigger \(\text{Ca}^{2+}\) release is so small and so local that it is not observable with current technology. The local apical \(\text{Ca}^{2+}\) release that actually activates the Cl\(^-\) channels in the apical membrane and the exocytotic enzyme release through the apical plasma membrane is therefore most likely the result of the final co-activation of IP\(_3\)Rs and RyRs triggered by the initial \(\text{Ca}^{2+}\) release from either IP\(_3\)Rs or NAADP-sensitive two-pore channels (Gerasimenko et al. 2015).

**Ca\(^{2+}\) tunnelling supports mid-range Ca\(^{2+}\) signalling in the Xenopus oocyte**

The *Xenopus* oocyte as an experimental model system to study Ca\(^{2+}\) signalling. The frog oocyte has long been a favoured model system to study Ca\(^{2+}\) signalling and has contributed significantly to our understanding of basic Ca\(^{2+}\) signalling mechanisms, including elementary Ca\(^{2+}\) release events, Ca\(^{2+}\) waves, fertilization-specific Ca\(^{2+}\) signals, biophysical properties of the IP\(_3\) receptor, and remodelling of Ca\(^{2+}\) signalling during the cell cycle (Lechleiter & Clapham, 1992; Sun et al. 1998; Bugrim et al. 2003; Foskett et al. 2007; Machaca, 2007). Several features make the oocyte an attractive model system to study these various aspects of Ca\(^{2+}\) signalling. The oocyte is large (~1.2 mm in diameter) allowing for easy spatial resolution of Ca\(^{2+}\) dynamics, which becomes particularly important for studies focused on the generation and propagation of Ca\(^{2+}\) waves, and elementary Ca\(^{2+}\) release events because their large spatial footprint in the oocyte makes them more amenable to investigation. The size of the oocyte also favours biochemical analyses and importantly linking them directly to Ca\(^{2+}\) signalling and other cell physiological processes at the single cell level (Machaca & Haun, 2002). Another unique advantage of the oocyte is the stage of the cell cycle oocytes transition through with two physiologically defined arrest points at prophase I and metaphase II of meiosis. Oocytes are arrested in prophase I at the G2/M transition of the cell cycle in a G2-like state, which is the typical stage in which they have been used as an experimental model. However, physiologically oocytes transition to metaphase of meiosis...
II in preparation for fertilization. This well-regulated progression through M-phase provides a distinctive window into the cell division phase of the cell cycle that is transient and asynchronous in other systems such as mitosis in mammalian cells, making it more difficult to study. Another additional benefit of the oocyte is the relative simplicity of its Ca\(^{2+}\) signalling toolkit compared to other cells. The frog oocyte has a limited well defined complement of Ca\(^{2+}\) channels and transporters, significantly less complex than most mammalian cells (Machaca, 2007).

Further simplifying Ca\(^{2+}\) signalling studies in the frog oocyte is the endogenous expression of Ca\(^{2+}\)-activated Cl\(^{-}\) channels (CaCCs), which are critical for oocyte biology and fertilization as they contribute significantly to the maintenance and regulation of the membrane potential. The Ca\(^{2+}\) transient generated at fertilization is initially localized at the site of sperm entry but gradually sweeps across the entire egg in the form of a Ca\(^{2+}\) release wave, which activates CaCCs and depolarizes the cell to prevent polyspermy (Machaca et al. 2001). This so called ‘fast electrical block’ to polyspermy in *Xenopus* is due to the fact that sperm–egg fusion is voltage sensitive in this species (Jaffe et al. 1983; Goul-Somero & Jaffe, 1984). The molecular entity underlying the CaCC in the frog oocyte has been identified as anoctamin 1 (Ano1) or TMEM16A ( Schroeder et al. 2008; Yang et al. 2008). The biophysical properties of the *Xenopus* oocyte CaCCs have been well characterized both for the endogenous current (Kuruma & Hartzell, 1998, 2000; Machaca & Hartzell, 1998, 1999; Callamaras & Parker, 2000), overexpressed Ano1 in the oocyte (Courjaret et al. 2016b), and heterologously expressed *Xenopus* Ano1 in the axolotl oocyte (Schroeder et al. 2008). In the oocyte, CaCC senses sub-cell membrane changes in Ca\(^{2+}\) concentration in real time and with high fidelity, whether this Ca\(^{2+}\) is released from the ER or flows from the extracellular space through channels in the plasma membrane (Machaca & Hartzell, 1999). As such multiple studies have used the endogenous CaCC to monitor complex Ca\(^{2+}\) dynamics mediated by endogenous or heterologously expressed Ca\(^{2+}\) permeable channels, such as ionotropic receptors (Kuruma & Hartzell, 1998), voltage-gated Ca\(^{2+}\) channels (Zhou et al. 2004), transient receptor potential (TRP) channels (Courjaret et al. 2013) and SOCE channels (Courjaret & Machaca, 2016).

**Mid-range Ca\(^{2+}\) signalling.** While studying the activation properties of CaCCs in the frog oocyte in response to various Ca\(^{2+}\) mobilizing agents, we noticed that CaCCs are stimulated to significantly higher levels when stores are depleted with IP\(_3\) as compared to other mobilizing agents that deplete Ca\(^{2+}\) stores by distinct mechanisms of action, including ionomycin, (N,N,N,N-tetrais(2-pyridylmethyl)-ethylenediamine (TPEN) and thapsigargin (Fig. 4A) (Courjaret & Machaca, 2014). While IP\(_3\) replicates the physiological situation, ionomycin, a Ca\(^{2+}\) ionophore with preferential insertion in the ER membrane ( Morgan & Jacob, 1994), empties Ca\(^{2+}\) stores rapidly and induces SOCE in the absence of activation of IP\(_3\) receptors (IP\(_3\)R). TPEN is a transition metal chelator with low Ca\(^{2+}\) affinity that is freely membrane permeant and chelates high luminal ER Ca\(^{2+}\), thus simulating Ca\(^{2+}\) store depletion and inducing

![Figure 4](https://example.com/figure4.png)
SOCE (Hofer et al. 1998), again in the absence of IP₃R activation. Thapsigargin is an irreversible, specific blocker of the endoplasmic reticulum Ca²⁺-ATPase (SERCA) (Thastrup et al. 1989) that leads to store depletion due to an inherent constitutive Ca²⁺ leak pathway from the ER. Therefore, blocking SERCA, the primary ER Ca²⁺ refilling pathway leads to store depletion, although with slower kinetics than other aforementioned Ca²⁺ mobilizing agents. Given the differing mechanisms by which these agents induce SOCE, it was not clear why IP₃ leads to a significantly higher induction of current through CaCCs. We ruled out an increased Ca²⁺ influx through SOCE under these various conditions (Courjaret & Machaca, 2014). Furthermore, we showed that Ca²⁺ transients in the cortical region of the oocyte were of similar amplitude when SOCE was stimulated with IP₃ or thapsigargin, but of much smaller amplitude with ionomycin (Fig. 4B) (Courjaret & Machaca, 2014). Given that measuring SOCE current reveals similar flow of Ca²⁺ into the cell, these data show that when SERCA is active, Ca²⁺ flowing through SOCE channels is immediately taken up by SERCA into the ER lumen, thus preventing its diffusion into the cortical region. When SERCA is blocked with thapsigargin, this pathway is inhibited allowing SOCE to flood the cell cortex with Ca²⁺. However, this does not explain why cortical Ca²⁺ is high during the Ca²⁺ influx phase when stores are depleted with IP₃ (Fig. 4B). Various approaches to block the IP₃R or SERCA were used to show that the high CaCC current in response to SOCE when IP₃ is present requires active IP₃Rs (Courjaret & Machaca, 2014). This led to the model outlined in Fig. 5, where Ca²⁺ flowing through SOCE channels is taken up within the SOCE microdomain by SERCA into the ER lumen and then released through open IP₃Rs at a distal site to specifically activate CaCCs, thus leading to high current levels specifically in response to SOCE (Fig. 5). This model matches the Ca²⁺ tunnelling mechanism discussed above in pancreatic acinar cells although the timing and functional links between the various components of the pathway diverge to meet the cell’s physiological needs.

Careful co-localization experiments in the oocyte confirm this model and show that Orai1, STIM1 and SERCA localize to the SOCE clusters at ER–PM junctions,
thereby creating a specialized Ca\textsuperscript{2+} handling domain that favours Ca\textsuperscript{2+} influx into the cytoplasm through Orai and uptake into the ER through SERCA (Courjaret \& Machaca, 2014). Importantly, in the case of CaCC as a downstream Ca\textsuperscript{2+} effector, store depletion is associated with a dramatic remodelling of the Ca\textsuperscript{2+} signalling machinery. STIM1, Orai1 and SERCA localize to SOCE clusters at ER–PM junctions. In contrast, Ano1 is excluded from these junctions and localizes to other areas of the plasma membrane (Fig. 6). At rest, Ano1 is evenly distributed throughout the cell membrane of the oocyte, including the dense brush of microvilli where it serves an additional scaffolding function and regulates microvilli length (Courjaret et al. 2016b). Store depletion, while concentrating STIM1, Orai1 and to a lesser extent SERCA into the SOCE clusters, excludes Ano1 resulting in the patchy separation illustrated in Fig. 6. In other words, there is little to no co-localization of the Ca\textsuperscript{2+} entry source (SOCE) and of the Ca\textsuperscript{2+} effector (CaCC), and the distance to be covered by Ca\textsuperscript{2+} ions in the cytoplasm from the mouth of the Orai channel to the CaCC is incompatible with a diffusion mechanism given the speed of activation of CaCCs and the measured size and distribution of SOCE puncta as compared to CaCC-rich membrane domains (Fig. 6). To overcome the diffusion barrier and reach the CaCCs, Ca\textsuperscript{2+} ions transit through the ER and are released at the target spot by IP\textsubscript{3}R. This spatial reorganization of the Ca\textsuperscript{2+} signalling machinery mediating SOCE and Ca\textsuperscript{2+} release results in the delivery of Ca\textsuperscript{2+} flowing into the cell through SOCE to a distal effector, CaCC, without inducing a global Ca\textsuperscript{2+} rise or having to contend with the limiting cytoplasmic Ca\textsuperscript{2+} diffusion. This signalling module allows for the transport of information carried by Ca\textsuperscript{2+} influx across distances that exceed the SOCE microdomain and are in the micrometre range or ‘mid-range’ between elementary and global signals.

Therefore, with SERCA active, the cytoplasmic Ca\textsuperscript{2+} transient within SOCE puncta at ER–PM junctions is transient and very localized, with the majority of the Ca\textsuperscript{2+} flux going into the ER and then leaking out at distal sites through IP\textsubscript{3}Rs to activate effectors with high specificity and efficiency. This is consistent with the small flux through Orai1 channels. Localization of SERCA to the STIM/Orai cluster is not restricted to Xenopus oocytes and has been reported in other cell types (Jouset et al. 2007; Sampieri et al. 2009; Alonso et al. 2012; Hogan, 2015). Therefore, following store depletion and the activation of SOCE, a pump–leak balance develops at the ER membrane with a point source pump pathway mediated by Orai1–STIM1–SERCA that is physically localized at SOCE puncta, and a leak pathway through open IP\textsubscript{3}Rs at distal sites to activate effectors such as CaCC (Fig. 5). We have previously proposed the term ‘Ca\textsuperscript{2+} teleporting’ to borrow an analogy from science fiction (Fort, 1931), to suggest rapid transport of Ca\textsuperscript{2+} through the ER lumen given the fact that ER stores are never fully depleted of Ca\textsuperscript{2+} (Courjaret et al. 2016a). Although a single Ca\textsuperscript{2+} ion is obviously not instantaneously traversing that distance through the ER lumen, the term teleporting nicely reflects the transfer of Ca\textsuperscript{2+} from the SOCE entry sites to CaCCs in a directed fashion to modulate CaCC current. In fact a direct physical interaction has been reported between the IP\textsubscript{3} receptor and Ano1 in neurons (Jin et al. 2013; Jin et al. 2014); whether a similar interaction exists in the oocyte remains unknown.

In addition to modulating the spatial aspects of Ca\textsuperscript{2+} signals and effector activation, Ca\textsuperscript{2+} tunnelling also modulates the temporal aspects of Ca\textsuperscript{2+} signals by favouring tonic over oscillatory Ca\textsuperscript{2+} signalling (Courjaret et al. 2016a). When Ca\textsuperscript{2+} stores are relatively full, IP\textsubscript{3} production favours Ca\textsuperscript{2+} oscillations resulting in repetitive transient Ca\textsuperscript{2+} signals. In contrast, when SOCE is fully activated with depleted Ca\textsuperscript{2+} store, Ca\textsuperscript{2+} tunnelling mediates pump–leak balance at the ER membrane that favours tonic sustained Ca\textsuperscript{2+} signalling but inhibiting Ca\textsuperscript{2+} oscillations (Courjaret et al. 2016a). In this case Ca\textsuperscript{2+}
Ca²⁺ tunnelling through the ER lumen targets the IP₃ receptor itself and modulates its properties to favour tonic rather than oscillatory signalling. Therefore, Ca²⁺ tunnelling not only modulates the spatial aspects of Ca²⁺ signalling, but also affects the temporal features of Ca²⁺ signals in the same cell. This has significant implications for encoding specific cellular responses downstream of SOCE using Ca²⁺ tunnelling.

**Is Ca²⁺ tunnelling active in other cells?**

The Ca²⁺ tunnelling system is clearly functional in pancreatic acinar cells and in the frog oocyte. Both of these cell types are highly specialized for a specific function, secretion in the acinar cell and fertilization and support of early development in the oocyte. Therefore, the question arises as to whether this Ca²⁺ signalling mode is ubiquitous or unique to some highly specialized cell types. We currently do not know the answer to this question, but several arguments support the conclusion that this Ca²⁺ signalling modality is widespread. First, SOCE is ubiquitous in non-excitable cells and present in excitable cells as well. SOCE is physiologically linked to IP₃ receptor activation downstream of agonist stimulation. Therefore, the entire machinery supporting Ca²⁺ tunnelling is present. The spatial remodelling of the Ca²⁺ signalling machinery in response to store depletion outlined in the oocyte system translates and has been described in other cells as well. Furthermore, the IP₃R has been shown to directly link to CaCC, a defined effector for Ca²⁺ tunnelling. Conceptually, the Ca²⁺ tunnelling mechanism is quite attractive as it allows for specific signalling to effectors through the SOCE pathway without inducing a global Ca²⁺ rise in the cytosol and without the need to localize multiple effectors into the physically limited space defined by ER–PM junctions where the SOCE machinery localizes.

In principle, Ca²⁺ tunnelling through the ER should be present in all cell types as it would seem unlikely that SERCA pumps and Ca²⁺ release channels should be exactly co-localized. Therefore Ca²⁺ would always tunnel a bit between Ca²⁺ uptake and release sites. The length of the effective tunnel would vary between cell types depending on their function. The effectiveness and speed of Ca²⁺ tunnelling would depend critically on the concentrations of Ca²⁺ buffers in the ER lumen, their mobility as well as their binding and dissociation rate constants. In addition, it would depend on the degree of depletion of ER Ca²⁺ stores. In the pancreatic acinar cells, it has been shown directly that there are only minor reductions in [Ca²⁺]ER during physiological stimulation (Park et al. 2000). In general, it is unlikely that there would be a need for complete depletion of ER Ca²⁺ stores before SOCE is activated. This is indeed the case in the frog oocyte, where IP₃-dependent release of Ca²⁺ from the ER fully activates SOCE without emptying the stores completely (Courjaret et al. 2016a).

In the ER lumen of the pancreatic acinar cells, the movement of Ca²⁺ immediately after localized uncaging of caged Ca²⁺ (following maximal ACh-induced Ca²⁺ release) has been directly monitored. The rate of rise of [Ca²⁺]ER decreases, as expected for a diffusional process, with increasing distance from the uncaging site (Park et al. 2000). At a distance of 10 μm from the uncaging site, the peak [Ca²⁺]ER occurs ~2.5 s later than at the uncaging site itself. Complete re-equilibration of [Ca²⁺] in the whole of the ER is attained 6–8 s after the uncaging event (Park et al. 2000). These data underestimate the speed of Ca²⁺ movement in the ER lumen under physiological conditions, because of the necessity of first having to evoke maximal release of Ca²⁺ from the ER in order to obtain a clear local increase in [Ca²⁺]ER upon Ca²⁺ uncaging. The free buffer concentration in these experiments (Park et al. 2000) would therefore have been higher than under more physiological conditions, where many of the buffers would already have been saturated with Ca²⁺.

One can readily postulate a long list of potential effectors that could be targeted by Ca²⁺ tunnelling with the most obvious being Ca²⁺-regulated ion channels located at the plasma membrane such as CaCCs, Ca²⁺-activated K⁺ channels (Liu et al. 1998), other integral membrane proteins such as adenylyl cyclases (Halls & Cooper, 2011), and Ca²⁺ sensitive enzymes anchored at the plasma membrane through A-kinase anchor proteins such as protein kinase C and phosphatase 2B (Esseltine & Scott, 2013). Ca²⁺ tunnelling effectors are likely to localize in the immediate vicinity of the release site, the IP₃R, and this can include virtually all the downstream effectors of the IP₃R that have been recently reviewed (Prole & Taylor, 2016). In the cytosol, organelles can also be a target for Ca²⁺ tunnelling, including lysosomes, nuclei, vesicles and mitochondria that can all localize next to IP₃Rs. Mitochondria are of particular interest given their intimate interaction with SOCE and the localization of IP₃R to ER-mitochondria junctions (Parekh, 2003).

Currently there are few validated targets of Ca²⁺ tunnelling including CaCCs, Ca²⁺-activated K⁺ channels, secretion in acinar cells, and the IP₃R itself where we have shown that Ca²⁺ tunnelling can modulate IP₃R activity switching it from a mode that favours Ca²⁺ oscillations to one that favours tonic Ca²⁺ signals (Courjaret et al. 2016a). There are also hints in the literature of potential additional effectors of Ca²⁺ tunnelling. In a human salivary gland cell line, the direct activation of the Ca²⁺-activated K⁺ channel by SOCE is limited by the fast buffering of Ca²⁺ below the plasma membrane and can be restored when the ER Ca²⁺ pump is inhibited by thapsigargin. When SOCE and IP₃ receptors are simultaneously activated (by stimulating muscarinic receptors with carbachol), Ca²⁺-sensitive K⁺ channels are strongly activated, supporting the idea that
SOCE ‘fuels’ the IP$_3$ receptors when the stores are empty to provide an efficient activation of the K$^+$ channel (Liu et al. 1998).

**Conclusion**

Herein we focus on findings from two distinctive specialized cell types, the pancreatic acinar cell and the frog oocyte, that led to proposing a novel model of Ca$^{2+}$ signalling that we refer to as Ca$^{2+}$ tunnelling. In pancreatic acinar cells, Ca$^{2+}$ tunnelling allows the transport of Ca$^{2+}$ flowing from the basolateral membrane to support transepithelial fluid transport and secretion of digestive enzymes. The tunnelling of Ca$^{2+}$ through the ER lumen circumvents the slow diffusion of Ca$^{2+}$ through the highly buffered cytosol and importantly delivers Ca$^{2+}$ to effectors in the apical membrane without inducing a global [Ca$^{2+}$], rise, which would undoubtedly activate multiple other Ca$^{2+}$-dependent processes. In oocytes, Ca$^{2+}$ tunnelling specifically and efficiently activates CaCCs downstream of SOCE without inducing a global Ca$^{2+}$ rise. This activation occurs spatially in the mid-range broader than the Ca$^{2+}$ microdomain but more contained than a global [Ca$^{2+}$], rise. This again eludes the need for Ca$^{2+}$ to diffuse long distances in the highly buffered cytosol and avoids a global [Ca$^{2+}$], rise while allowing the activation of a specific effector, CaCC, downstream of SOCE. In addition, Ca$^{2+}$ tunnelling in the oocyte modulates the spatial features of Ca$^{2+}$ signals favouring a tonic signal while inhibiting Ca$^{2+}$ oscillations by acting on the IP$_3$R itself, in this case as a downstream effector.

Of note is the mechanism underlying Ca$^{2+}$ tunnelling with SOCE forming the Ca$^{2+}$ entry pathway that fuels the whole process. Ca$^{2+}$ entering the cell within the SOCE microdomain is unlikely to diffuse beyond the microdomain due both to the cytoplasmic Ca$^{2+}$ buffering and also to the rapid uptake into the ER lumen through the action of SERCA. This is somewhat reminiscent of the capacitative Ca$^{2+}$ entry model originally proposed by Jim Putney (Putney, 1986), where it was postulated that Ca$^{2+}$ enters the cell directly from the extracellular space into the ER lumen. Although it is now clear that this is not the case, the limited diffusion of Ca$^{2+}$ beyond the SOCE microdomain and the rapid uptake of Ca$^{2+}$ flowing through SOCE into the ER lumen argue that a significant proportion of the signalling downstream of SOCE occurs through Ca$^{2+}$ tunnelling.

Interestingly, the molecular mechanisms underlying Ca$^{2+}$ tunnelling in acinar cells and oocytes are analogous. The machinery mediating Ca$^{2+}$ tunnelling encompasses STIM1 and Orai1 (SOCE), the SERCA pump and the IP$_3$R. Store depletion stabilizes the STIM1–Orai1 puncta at ER–PM junctions thus providing the source for Ca$^{2+}$ entry from the extracellular space. Ca$^{2+}$ flowing through SOCE channels is taken up by the SERCA pump into the ER lumen preventing its diffusion out of the SOCE microdomain. In turn ER Ca$^{2+}$ is released through IP$_3$Rs thus delivering it to the appropriate effectors (secretion, CaCC, IP$_3$R) with high efficiency and specificity. The Ca$^{2+}$ tunnelling machinery has been adapted to very different cell physiological needs in the oocyte as compared to the pancreatic acinar cell. In the oocyte it modulates resting membrane potential and the temporal features of Ca$^{2+}$ signals, whereas in the acinar cell it drives enzyme secretion and fluid flow. Given that the molecular machinery underlying Ca$^{2+}$ tunnelling is ubiquitous, it is likely that this pathway is involved in Ca$^{2+}$ signalling in a plethora of other physiological functions. The remarkable functional link between SOCE, SERCA and IP$_3$R conscripted to allow Ca$^{2+}$ tunnelling results in the delivery of Ca$^{2+}$ to effectors that could easily be missed experimentally and interpreted as signalling downstream of SOCE directly. It is therefore likely that Ca$^{2+}$ tunnelling activates additional cell physiological events that remain to be defined.

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**Additional information**

**Competing interests**

None declared.

**Author contributions**

All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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