Aberrant localization of intracellular organelles, Ca\textsuperscript{2+} signaling and exocytosis in Mist1 Null Mice

Xiang Luo\textsuperscript{1,4}, Dong Min Shin\textsuperscript{2,4}, Xinhua Wang\textsuperscript{1}, Stephen F. Konieczny\textsuperscript{3} and Shmuel Muallem\textsuperscript{1,5}

From the \textsuperscript{1}Department of Physiology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75390-9040, \textsuperscript{2}The Department of Oral Biology, Research Center for Orofacial Hard Tissue Regeneration, Brain Korea 21 Project for Medical Science, Yonsei University College of Dentistry, Seoul 120-752, Korea and \textsuperscript{3}The Department of Biological Sciences and the Purdue Cancer Center, Purdue University, 201 S. University St., West Lafayette, IN 47907-2064

\textsuperscript{4}These authors contributed equally to this work and should be considered first co-authors

\textsuperscript{5}Address for correspondence:
Dr. Shmuel Muallem
The University of Texas Southwestern Medical Center at Dallas
5323 Harry Hines Boulevard
Dallas, TX 75390-9040
Telephone: 214-648-2593
Fax: 214-648-2974
Email: SHMUEL.MUALLEM@utsouthwestern.edu

Short Title: Ca\textsuperscript{2+} signaling and exocytosis in Mist1\textsuperscript{–/–} cells

Abbreviations used:
GPCRs, G proteins coupled receptors; CCKR, CCK receptors; M3R, M3 muscarinic receptors; IP\textsubscript{3}, inositol 1,4,5 trisphosphate; Man II, Mannosidase II; pNPP, \textit{para}-pitrophenyl palmitate; BApNA, N-a-benzoyl-arginine-p-nitroanilide; PMCA, plasma membrane Ca\textsuperscript{2+} ATPase pump; SERCA2b, sarco/endoplasmic reticulum Ca\textsuperscript{2+} ATPase pump; ER, endoplasmic reticulum; TMRM, Tetramethylrhodamine methyl ester; PMA, Phorbol 12-myristate 13-acetate; FCCP, carbonyl cyanide \textit{p-}(trifluoromethoxy)phenylhydrazone.
Abstract

Ca²⁺ signaling and exocytosis are highly polarized functions of pancreatic acinar cells. The role of cellular architecture in these activities and the capacity of animals to tolerate aberrant acinar cell function are not known. A key regulator of acinar cell polarity is Mist1, a bHLH transcription factor. Ca²⁺ signaling and amylase release were examined in pancreatic acini of WT and Mist1 null mice to gain insight into the importance of cellular architecture for Ca²⁺ signaling and regulated exocytosis. Mist1⁻/⁻ acinar cells exhibited dramatically altered Ca²⁺ signaling with up-regulation of CCK receptor but minimal effect on expression of the M3 receptor. However, stimulation of IP₃ production by CCK and carbachol was inefficient in Mist1⁻/⁻ cells. Although agonist stimulation of Mist1⁻/⁻ cells evoked a Ca²⁺ signal, often the Ca²⁺ increase was not in the form of typical Ca²⁺ oscillations, but rather in the form of a peak-plateau type response. Mist1⁻/⁻ cells also displayed distorted apical-to-basal Ca²⁺ waves. The aberrant Ca²⁺ signaling was associated with mis-localization and reduced Ca²⁺ uptake by the mitochondria of stimulated Mist1⁻/⁻ cells. Deletion of Mist1 also led to mis-localization of the Golgi apparatus and markedly reduced digestive enzyme content. The combination of aberrant Ca²⁺ signaling and reduced digestive enzyme content resulted in poor secretion of digestive enzymes. Yet, food consumption and growth of Mist1⁻/⁻ mice were normal for at least 32 weeks. These findings reveal that Mist1 is critical to normal organelle localization in exocrine cells and highlight the critical importance of maintaining cellular architecture and polarized localization of cellular organelles in generating a propagating apical-to-basal Ca²⁺ wave. The studies also reveal the spare capacity of the exocrine pancreas that allows normal growth and development in the face of compromised exocrine pancreatic function.
Introduction

Ca\(^{2+}\) signaling regulates virtually all cell functions, including long-term functions such as transcription and translation, and short-term functions such as neurotransmission and exocytosis [1, 2]. In the pancreas, activation of Ca\(^{2+}\) signaling by G protein coupled receptors (GPCRs) plays a central role in digestive enzyme secretion [3]. Abnormal enzyme secretion can lead to numerous diseases, including malnutrition in Cystic Fibrosis [4] and, more commonly, acute pancreatitis [5]. Several studies have shown a strong association between aberrant Ca\(^{2+}\) signaling and pancreatitis [6-8]. The polarized function of exocrine secretory cells requires polarized Ca\(^{2+}\) signaling. Indeed, stimulation of pancreatic acini GPCRs with physiological agonist concentrations triggers repetitive [Ca\(^{2+}\)]\(_i\) oscillations in the form of Ca\(^{2+}\) waves that initiate at the apical pole and propagate to the basal pole [9-13].

The apical-to-basal Ca\(^{2+}\) waves are achieved by clustering Ca\(^{2+}\) signaling complexes at the apical pole [14-16]. This leads to an apical-to-basal gradient of responsiveness, with the most responsive GPCR Ca\(^{2+}\) signaling complexes at the apical pole [16]. In fact, functional mapping of Ca\(^{2+}\) signaling complexes has revealed that physiological Ca\(^{2+}\) signals are mostly triggered by stimulation of Ca\(^{2+}\) signaling complexes at the apical pole [16]. Once launched, the shape of the Ca\(^{2+}\) signals are regulated by many processes that affect the biochemical component of the Ca\(^{2+}\) signal that generates 1,4,5-trisinositolphosphate (IP\(_3\)) and the biophysical component that includes Ca\(^{2+}\) release and influx channels and sarco/endoplasmic reticulum ATPase (SERCA) and plasma membrane Ca\(^{2+}\) ATPase (PMCA) pumps [16].

Another important regulator of Ca\(^{2+}\) signaling in all cells [17], including pancreatic acinar [18, 19] and other secretory cells [20], are mitochondria. The seminal work of Rizzuto and Pozzan showed that the mitochondria are in close proximity to the ER [21], thereby aligning the mitochondrial Ca\(^{2+}\) uptake pathway with the IP\(_3\)Rs to incorporate a large portion of the Ca\(^{2+}\) released from the ER [22, 23]. Subsequently, mitochondria were shown to communicate with I\(_{crae}\) channels to regulate Ca\(^{2+}\) influx across the plasma membrane [24, 25]. Mitochondrial localization in pancreatic acini is even more intricate. In these cells three populations of energized mitochondria have been identified; a belt capping the secretory granules, a ring surrounding the nucleus and a string lining the plasma membrane [19]. The belt capping the secretory granules functions to confine Ca\(^{2+}\) signals to the apical pole at very low stimulus
intensity [18], while the ring surrounding the nucleus isolates the nucleus from cytosolic Ca\(^{2+}\) tides [15, 19].

The intricate localization of Ca\(^{2+}\) signaling complexes and intracellular organelles is likely to be critical for the precise operation of the Ca\(^{2+}\) signaling apparatus and for regulated exocytosis in acinar cells. These assumptions can be directly examined only by testing the effect of perturbation of the cellular architecture on pancreatic acinar cell function. This has been a difficult problem to address, but has now become possible with the availability of Mist1 null (Mist1\(^{-/-}\)) mice [26]. Mist1 is a basic helix-loop-helix transcription factor that is essential for the normal development of serous acinar cells in various secretory glands, including the pancreas and salivary glands [26-30]. Deletion of the Mist1 gene [26, 30] or inhibition of Mist1 function [28, 29] leads to severe distortion of acinar cellular architecture, including loss of gap junctions and intercellular communication [28, 30], disorder of secretory granules [26-30] and acinar-to-ductal metaplasia [29]. Additional changes in Mist1\(^{-/-}\) cells include up-regulation of mRNA coding for the cholecystokinin (CCK) receptors and down-regulation of expression of IP\(_3\)R3 [26]. However, the effect of distorted cellular architecture on Ca\(^{2+}\) signaling, pancreatic function and animal growth and development has not been examined. In the present work, we show that the Ca\(^{2+}\) signaling machinery in Mist1\(^{-/-}\) cells is dramatically altered, resulting in aberrant agonist-evoked Ca\(^{2+}\) oscillations and Ca\(^{2+}\) waves. The altered Ca\(^{2+}\) response is not due to mis-localization of the remaining IP\(_3\) receptors, but rather is a consequence of mis-localization and aberrant Ca\(^{2+}\) uptake into mitochondria. The Golgi apparatus of Mist1\(^{-/-}\) cells is diffuse and fragmented, which may explain the markedly decreased digestive enzyme content in the secretory granules. Exocytosis by Mist1\(^{-/-}\) acinar clusters is undetectable at physiological agonist concentrations and is greatly reduced at pharmacological agonist concentrations. Remarkably, food consumption and weight gain remain similar for WT and Mist1\(^{-/-}\) mice. These findings provide experimental evidence for the importance of cellular architecture for the generation and propagation of Ca\(^{2+}\) signals and illustrate the spare capacity of the exocrine pancreas to allow normal growth at reduced exocytosis.
Materials and Methods

**Materials:** Carbachol, Cholecystokinin (CCK) and Phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma. Inositol 1,4,5-trisphosphate (IP$_3$) was from Alexis. Fura2/AM, mitotracker green, Rhod-2, and Fluo-3 were from Teff Labs. Tetramethylrhodamine methyl ester (TMRM) was from Molecular probes. Dr. Akihiko Tanimura (University of Hokkaido, Japan) generously provided anti-IP$_3$R1, IP$_3$R2, and IP$_3$R3 pAb. Anti-PMCA mAb 5F10 was purchased from Affinity Bioreagents. Anti-Man II pAbs were obtained from the University of Georgia. pAb against SERCA2b was provided by Dr. Frank Wuytack (University of Leuven, Belgium). Anti-M3 receptors antibodies were from USBiological.

**Experimental animals, body weights and food intake:** Mist1$^{-/-}$ mice were generated as described previously (26). All animals were housed in the animal care facility and all protocols were approved by The University of Texas Medical Center at Dallas Animal Care Committee, and in accordance with the NIH Guide for Care and Use of Laboratory Animals. WT and Mist1$^{-/-}$ mice were individually housed. Food intake was measured three times per week using an Ohaus portable electronic scale with a sensitivity of 0.1 g. Body weight of littermates was measured on the day of birth and then at one week intervals.

**Preparation of pancreatic acini:** Pancreatic acini and small acinar clusters were prepared by collagenase digestion as detailed previously [31]. After isolation, the cells were suspended in solution A (in mM: 140 NaCl, 5 KCl, 1 MgCl$_2$, 1 CaCl$_2$, 10 HEPES (pH 7.4 with NaOH), 10 glucose, 0.1% bovine serum albumin, and 0.02% soybean trypsin inhibitor) and kept on ice until used.

**[Ca$^{2+}$]$_i$ imaging:** Pancreatic acinar cells were loaded with Fura2, and [Ca$^{2+}$]$_i$ was imaged as described [32]. In brief, to measure the dose response for agonists, Fura2 fluorescence ratio was measured at excitation wavelengths of 350 and 380 nm and the ratio was calibrated to obtain [Ca$^{2+}$]$_i$. Ca$^{2+}$ waves were recorded using a single excitation wavelength of 380 nm. The image of resting cells was acquired and was taken as the fluorescence signal at time 0 ($F_0$). All subsequent images were divided by this image, and the traces and images are the calculated $F_t/F_0$, where $F_t$ is the fluorescence at time $t$.

**Simultaneous measurement of cytosolic and mitochondrial Ca$^{2+}$:** Rhod-2 and Fluo-3 were used for measurement of mitochondrial and cytosolic Ca$^{2+}$ signals, respectively. Loading with
Rhod-2 was achieved by incubating acini in solution A with 8 µM Rhod-2/AM at 4 °C for 15 min. The acini were washed by centrifugation for 2 min at 30g to remove excess dye and resuspended in solution A. The acini were incubated at room temperature for 30 min to hydrolyze the trapped Rhod-2. During this incubation the acinar suspension was supplemented with 4 µM Fluo-3/AM. Finally, the acini were washed with solution A and kept on ice until use. Dye-loaded cells were transferred to a perfusion chamber, and Fluo-3 and Rhod-2 fluorescence was measured with a confocal laser-scanning system from Bio-Rad (MRC-1024) using the 488 and 568 lines, respectively. Laser intensity was reduced to 1-3% with neutral density filters to reduce photo bleaching. Images were recorded at a frequency of 1 Hz. The images were analyzed using MetaMorph software.

**Mitochondrial membrane potential:** Mitochondrial membrane potential was estimated from the incorporation of the potential sensitive dye TMRM. Preliminary experiments showed that when monitored continuously, even at 1 µM the extent of TMRM incorporation was proportional to the mitochondrial membrane potential. TMRM uptake was measured by continuously perfusing acinar cells with a solution containing 1 µM TMRM. Once a plateau was reached the acini were perfused with dye-free medium and then with medium containing 10 µM FCCP. TMRM fluorescence was recorded with the 488 line of the Bio-Rad MRC-1024 confocal microscope.

**Measurement of 1,4,5-IP₃:** IP₃ levels were measured by a radioligand assay as described elsewhere [33]. Acini suspended in solution A and incubated at 37°C were stimulated with the indicated CCK or carbachol concentrations for 5-15 seconds, depending on agonist concentration. The reactions were stopped by addition of perchloric acid, vigorous mixing, and incubation on ice for at least 10 min to allow precipitation of proteins. The supernatants were collected and transferred to clean tubes. Standards of IP₃ were prepared in the same manner. The perchloric acid was removed and IP₃ extracted by the addition of 0.15 ml of Freon and 0.15 ml of tri-n-octylamine. IP₃ content in the aqueous phase was measured by displacement of [³H]IP₃ using microsomes prepared from bovine brain cerebella.

**RT-PCR analysis of CCK and M3 receptors expression:** Acinar cell digests were placed in a Petri dish and small clusters consisting of 3-5 cells were collected with a Pasteur pipette under microscopic examination to ensure lack of contamination with other cell types. The RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA) from the acinar cells and brains of WT
and Mist1−/− mice and dissolved in DEPC-treated water. RT-PCR was performed using the same amount of RNA isolated from 3 WT and 3 Mist1−/− mice. The RT reaction was performed with the Superscript™ II RT kit (Invitrogen) in a 20-µl reaction volume as suggested by the manufacturer. PCR primers were designed using Primer 3 version 0.2 and alignment by NCBI Blast software. The primer sequences used were as follows: CCKA Receptor-sense, tcagtgacctcatgctttgc; antisense, atgagtccgtaagccaccac; size of PCR product, 442 bp; Muscarinic receptor 3- sense, tgcctggtgcacggtgag; antisense, ttcctgtgctgctgttgtag; size of PCR product, 427 bp; β-actin-sense, tgttaccaactgggacgaca; antisense, ttcagctggtgaag; size of PCR product, 392 bp. The PCR reaction was initiated by a 5 min hot start at 94°C followed by 35 amplification cycles that consisted of 50 sec incubation at 94°C, 1 min at 55°C, 1 min at 72°C and extended by 10 min at 72°C and terminated by incubation at 4°C. Preliminary experiments using different amounts of cDNA were used to determine the optimal conditions and amplification of actin mRNA was used to calibrate between samples.

**Immunoblotting:** Brain microsomes were prepared by homogenizing brain tissue from WT and Mist1−/− mice in a buffer containing (in mM, pH 7.6 with KOH) 100 KCl, 20 Tris-base, 1 EDTA, 1 benzamidine and 1 PMSF. The homogenate was centrifuged at 1000g for 10 min at 4°C. The supernatant was collected and centrifuged at 40,000g for 30 min. The pellet was resuspended in homogenization buffer and the microsomes were stored at -80°C until use. The microsomes were extracted by 1 hr incubation on ice with a buffer containing (in mM) 50 Tris (pH 6.8 with HCl), 150 NaCl, 2 EDTA, 2 EGTA and 0.5% Triton X-100 supplemented with protease inhibitors (0.2 mM PMSF, 10 µg/ml leupeptin, 15 µg/ml aprotinin, and 1 mM benzamidine). Released proteins were separated by SDS-PAGE, transferred to 0.2 µm polyvinylidene difluoride membranes, and the membranes were blocked by 1 hr incubation at room temperature in 5% nonfat dry milk in a solution containing 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.05% Tween 20 (TTBS). The IP3R1, IP3R2, IP3R3, PMCA, SERCA2b and M3 receptors were detected by a 1-2-hr incubation of individual membranes with the respective antibodies diluted in TTBS.

**Immunocytochemistry:** Immunostaning was performed as detailed before [31, 32]. Frozen pancreatic sections were fixed and permeabilized with 0.5 ml of cold methanol for 10 min at -20°C. The sections were washed with PBS alone, PBS containing 50 mM glycine and the nonspecific sites were blocked by 1 hr incubation with PBS containing 5% goat serum, 1%
bovine serum albumin, and 0.1% gelatin. The medium was aspirated and replaced with 50 µl of blocking medium containing control serum or 1:100 dilution of antibodies against M3R, IP_3R2 and IP_3R3, and 1:1000 dilution of antibodies against Man II. After incubation with the primary antibodies overnight at 4°C and three washes with the incubation buffer, the antibodies were detected with goat anti-rabbit or anti-mouse IgG tagged with fluorescein or rhodamine. Images were collected with a Bio-Rad MRC 1024 confocal microscope.

**Measurement of amylase, lipase and trypsin:** Freshly isolated acini from one mouse were suspended in 50 ml (for amylase) or 15 ml (for lipase and trypsin) of solution A. To measure total enzyme activity 1 ml of cell suspension was lysed by addition of 1% Triton X-100, centrifuged for 2 min at 2000g at 4°C and the supernatant collected. A second sample was used to measure protein content. Enzyme activity was measured in parallel from WT and Mist1^{-/-} mice. To reduce variation due to feeding schedule the mice were fasted for 24 hr before preparation of acini and the results are expressed as the ratio between the activities in WT/Mist1^{-/-} cells. For measurement of exocytosis, portions of the 1.5 ml cell suspension were transferred to vials containing agonists to give the desired final concentrations. After 30 min incubation at 37°C, samples were transferred to Eppendorf tubes, the supernatants were separated from the acini by centrifugation and amylase released to the medium was measured. In each experiment, samples of cells were lysed to measure the total amylase content and exocytotic amylase release was calculated as a fraction of total amylase content.

Amylase activity was measured with a Phedabase kit (Pharmacia & Upjohn 10-5380-33) as described previously [34]. In brief, 10 µl samples were diluted into 200 µl buffer containing 20 mM NaH_2PO_4, 20 mM Na_2HPO_4, 50 mM NaCl and 0.02% NaN_3, pH 7.0. 20 µl of the diluted samples were mixed with 1 ml blue starch (10 mg/ml) and incubated for 10 min at 37°C with gentle shaking. The reaction was stopped by addition of 250 µl of 2 M NaOH, the supernatant was cleared by 5 min centrifugation at 14,000 rpm and the absorbance measured at a wavelength of 595 nm.

Lipase activity was measured using para-pitrophenyl palmitate (pNPP) as a substrate [35]. Samples of 0.1 ml were added to 2.4 ml of a freshly prepared pNPP solution (30 mg pNPP in 100 ml 100 mM Tris buffer, pH 8.5, 207 mg Na^{+}-Deoxycholate and 100 mg Gum Arabic) and incubated for 1 hr at 37°C with gentle shaking. The reactions were terminated by addition of 0.2
ml 100 mM CaCl₂, the samples centrifuged at 14,000 rpm for 2 min and the absorbance was measured at a wavelength of 410 nm.

Trypsin activity was determined with the substrate N-a-benzoyl-arginine-p-nitroanilide (BAPNA) as described before [36] with a slight modification. Samples of 100 µl were added into 2.4 ml of 1 mM BAPNA dissolved in a solution containing 100 mM Tris, pH 9.0 and 10 mM CaCl₂ and incubated for 1 h at 37°C. The reactions were stopped by addition of 5 µl of 0.5 mg/ml soybean trypsin inhibitor and the change in absorbance at 410 nm was monitored.

**Statistics:** When appropriate, results are presented as the mean ± S.E.M. of the indicated number of experiments. Statistical significance was evaluated by a two way ANOVA. All immunostaining experiments were repeated at least five times with similar results.
Results and Discussion

**Impaired stimulation of Ca$^{2+}$ signaling in Mist1$^{-/-}$ cells:** The up-regulation of the CCK receptors (CCKRs) mRNA in Mist1$^{-/-}$ mouse pancreatic acinar cells [26] was confirmed by RT-PCR in pancreatic acinar cells (Figure 1A). However, this increase was not observed for all GPCRs. Initial analysis by RT-PCR showed that expression of the M3 receptors (M3Rs) mRNA in the pancreas of Mist1$^{-/-}$ mice was unchanged or slightly up-regulated (Figure 1A). An attempt to quantitate the extent of protein expression of M3Rs by Western blot in pancreatic extracts failed due to poor signal/noise. However, the antibodies gave a reasonable signal in immunostaining. Figures 1B and 1C show enrichment of M3Rs expression at the apical pool of cells from WT and Mist1$^{-/-}$ mice and comparable staining intensity. To further analyzed receptors expression we examined expression of mRNA and protein in the brain of the mice. Figure 1D shows that, similar to findings in the pancreas, brain mRNA for CCKRs was up-regulated and for M3Rs was unchanged. Western blot analysis of extracts prepared from 5 brains of WT and 5 brains of Mist1$^{-/-}$ mice showed a small reduction in the level of M3Rs protein in Mist1$^{-/-}$ mice (18±5%, n=5) (Figure 1E).

The aberrant localization of secretory granules, the reduction in expression of IP$_3$R3 and up-regulation of the CCKRs mRNA in the Mist1$^{-/-}$ mouse pancreatic acini [26] raised the question of how Ca$^{2+}$ signaling is affected in these cells. Measurement of IP$_3$ production showed that signaling by all GPCRs was impaired in Mist1$^{-/-}$ cells. Loss of Mist1 protein reduced the EC$_{50}$ for CCK stimulation from about 0.83 to 0.032 nM, and reduced the maximal production of IP$_3$ by about 50% (Figure 2A). Mist1$^{-/-}$ acinar cells showed an increased EC$_{50}$ for carbachol from about 3.7 to 46 µM and a reduced maximal production of IP$_3$ of about 30% (Figure 2B). The increased apparent affinity to CCK may relate to the increased mRNA levels of the CCKRs [26, Figure 1A]. The modest change in M3R mRNA and protein is consistent with this interpretation. However, the reduction in maximal IP$_3$ production indicates a general impaired activation of G proteins by the M3R and an impaired activation of phospholipase Cβ by GPCRs.

The consequence of impaired IP$_3$ production on the pattern of Ca$^{2+}$ signaling is shown in Figures 3 and 4. Panels (A) and (B) in each figure show example traces from individual experiments whereas panels (C) summarize results from multiple experiments. CCK stimulated Ca$^{2+}$ signaling with a higher affinity in Mist1$^{-/-}$ cells (Figure 3C), whereas the affinity for
carbachol to trigger a Ca\(^{2+}\) signal was lower in Mist1\(^{-/-}\) cells (Figure 4C), as expected from their effects on IP\(_3\) production. However, maximal concentrations of both agonists increased [Ca\(^{2+}\)]\(_i\) to similar levels in WT and Mist1\(^{-/-}\) cells (CCK: WT, 723±81, Mist1\(^{-/-}\), 744±79 nM; Carbachol: WT, 726±88, Mist1\(^{-/-}\) 711±63 nM, n>35 acini from 11 Mist1\(^{-/-}\) and 11 age-matched WT mice, ages 2-11 months). A notable difference between WT and Mist1\(^{-/-}\) cells was that often agonists did not induce [Ca\(^{2+}\)]\(_i\) oscillations in the Mist1\(^{-/-}\) cells but rather a single Ca\(^{2+}\) transient with a subsequent plateau (Figures 3A,B; 4A,B).

The Ca\(^{2+}\) signal in pancreatic acinar cells occurs as a propagated Ca\(^{2+}\) wave [9-13]. Therefore, we measured Ca\(^{2+}\) waves in WT and Mist1\(^{-/-}\) cells. Stimulation of WT acini with low agonist concentrations such as 1 \(\mu\)M carbachol (not shown) or 10 pM CCK (Figure 5A) resulted in a slowly propagating Ca\(^{2+}\) wave that was initiated at the apical pole. On the other hand, in multiple attempts it was almost impossible to resolve a Ca\(^{2+}\) wave at low agonist concentrations in Mist1\(^{-/-}\) cells (for example, Figure 5B). However, we noted that [Ca\(^{2+}\)]\(_i\) increased faster in Mist1\(^{-/-}\) cells. The Ca\(^{2+}\) waves in Mist1\(^{-/-}\) cells could sometimes be resolved at intermediate agonist concentrations. Stimulation of heterozygous Mist1\(^{+/-}\) cells (as well as WT cells) with 5 \(\mu\)M carbachol generated Ca\(^{2+}\) waves that propagated through the cell at a rate of 16.7±0.5 \(\mu\)m/sec (Figure 5C). Interestingly, although Mist1\(^{-/-}\) cells also initiated a Ca\(^{2+}\) signal at the apical pole, even at the intermediate agonist concentrations it was not always possible to observe a clear Ca\(^{2+}\) wave for two main reasons (Figure 5B). First, the Ca\(^{2+}\) wave in Mist1\(^{-/-}\) cells propagated very rapidly. When it could be resolved, the Ca\(^{2+}\) wave propagated at a rate of 43.6±5.8 (n=14) \(\mu\)m/sec. Second, the Ca\(^{2+}\) wave in Mist1\(^{-/-}\) cells propagated along the cell periphery and only then proceeded concentrically towards the cell center (Figure 5D). These results suggest that the proteins and organelles that control the propagation of the Ca\(^{2+}\) wave do not function properly in the Mist1\(^{-/-}\) cells. This can be mis-localization of IP\(_3\)Rs that are concentrated at the apical pole of pancreatic acini [14, 15], mis-localization of the mitochondria that determine the pattern of the Ca\(^{2+}\) wave [18-20], or both.

**Expression and localization of Ca\(^{2+}\) signaling proteins:** The Ca\(^{2+}\) waves in pancreatic acini are regulated by localization of IP\(_3\) receptors [14, 15] and other Ca\(^{2+}\) signaling proteins [13, 16] and by Ca\(^{2+}\) uptake into the mitochondria [18-20]. Our previous work has shown that the level of IP\(_3\)R3 is down-regulated in Mist1\(^{-/-}\) cells [26]. To test if down-regulation of IP\(_3\)R3 is a more general phenomenon, and whether deletion of the *Mist1* gene affects localization of Ca\(^{2+}\)
signaling complexes, we analyzed expression of individual IP3Rs in brain extracts and localization of IP3R2 and IP3R3 in pancreatic acini. The western blot analysis in Figure 6A shows that deletion of Mist1 reduced expression of IP3R3 in the brain by about 65±11% (n=5). On the other hand, expression of IP3R1, IP3R2, SERCA2b and PMCA was not affected. This indicates that down-regulation of IP3R3 expression in Mist1−/− mice is not specific to acinar cells, raising the possibility that loss of Mist1 may effect IP3R3 expression in other cell types. The immunolocalization in Figure 6B revealed lack of IP3R3 and completely normal localization of IP3R2 in pancreatic acini of Mist1−/− cells.

The results in Figure 6 indicate that the distorted Ca2+ signaling and Ca2+ waves observed in Mist1−/− cells are not due to a general aberrant expression of Ca2+ signaling proteins or due to mis-localization of the remaining IP3Rs. Furthermore these finding and those in Figures 3 and 4 indicated that the function of IP3R3, and perhaps other IP3Rs, is cell specific. A recent work provided strong evidence in cell lines that IP3R1 is essential for receptor-triggered Ca2+ oscillations whereas IP3R3 functions as an anti-Ca2+ oscillatory unit [38]. That is, knock-down of IP3R3 by siRNA enhanced Ca2+ oscillations. On the other hand, Ca2+ oscillations were rare in Mist1−/− cells where the IP3R3 is markedly down-regulated (Figures 3, 4). This would suggest a cell specific function of IP3R3.

**Mitochondrial localization and function in Mist1−/− cells:** The Ca2+ waves in pancreatic acini and Ca2+ signaling in many cells are prominently regulated by Ca2+ uptake into the mitochondria [18-25]. Therefore, we next compared localization and function of the mitochondria in WT and Mist1−/− cells. Localization of mitochondria was followed using mitotracker green detection and mitochondrial and cytosolic Ca2+ levels were measured with Rhod-2 and Fluo 3, respectively. As expected, the energized mitochondria in WT cells were clustered around the secretory granules, the nucleus and next to the plasma membrane, but were completely excluded from the secretory granule area in pancreatic acini (Figures 7A, 7B, 7E) [19]. By contrast, mitochondria in Mist1−/− acinar cells were highly disorganized (Figures 7C, 7D, 7F). Energized mitochondria were found at all regions of the basal pole. In addition, although mostly excluded from the apical pole in Mist1−/− cells, some mitochondria could also be found at the periphery of the apical pole or sometimes within the apical pole itself.

Disorganization of the mitochondria in Mist1−/− cells resulted in inhibition of Ca2+ uptake into the mitochondria when the cells were stimulated with low agonist concentrations. In WT
cells $[\text{Ca}^{2+}]_i$ and mitochondrial $\text{Ca}^{2+}$ increases were observed when the cells were stimulated with carbachol concentrations between 0.1-1 $\mu$M. By contrast, a $[\text{Ca}^{2+}]_i$ increase was observed at carbachol concentrations of 0.5 and 1 $\mu$M in Mist1$^{-/-}$ cells with no change in mitochondria $\text{Ca}^{2+}$. Figures 7G and 7H show the response to 0.5 $\mu$M carbachol. However, a normal increase in mitochondrial $\text{Ca}^{2+}$ was observed at carbachol concentrations above 5 $\mu$M. The nearly normal increase in mitochondrial $\text{Ca}^{2+}$ at intermediate agonist concentrations suggests that mis-localization of mitochondria rather than loss of mitochondrial membrane potential was the major reason for reduced $\text{Ca}^{2+}$ uptake into the mitochondria at low agonist concentration. This was verified directly by measuring the mitochondrial membrane potential with TMRM. Figures 7I and 7J show the similar TMRM uptake in WT and Mist1$^{-/-}$ cells, respectively.

Mitochondrial localization has a critical role in $\text{Ca}^{2+}$ signaling. Previous work has emphasized the importance of the close apposition of the mitochondria to the ER for $\text{Ca}^{2+}$ uptake into the mitochondria [22, 23], regulation of the $I_{\text{crac}}$ $\text{Ca}^{2+}$ influx channel [24, 25] and regulation of the $\text{Ca}^{2+}$ wave in secretory cells [18-20]. Furthermore, $\text{Ca}^{2+}$ uptake into the mitochondria is essential for mitochondrial and cellular energy metabolism [39, 40]. The disorganization of the mitochondria in Mist1$^{-/-}$ cells can explain the lack of $\text{Ca}^{2+}$ uptake by the mitochondria when the cells are stimulated at low agonist concentration. Such an uptake requires close communication between the mitochondria and the ER [21, 23]. Since $\text{Ca}^{2+}$ uptake by the mitochondria plays an important role in controlling the $\text{Ca}^{2+}$ wave [18-20], the disorganization of mitochondria is likely responsible for the rapid spreading of $\text{Ca}^{2+}$ from the apical to the basal pole and the concentric $\text{Ca}^{2+}$ signal observed in Mist1$^{-/-}$ cells (Figure 5).

Digestive enzyme content in Mist1$^{-/-}$ cells: To determine the effect of deleting the Mist1 gene on pancreatic exocrine function it was necessary to first measure how the absence of Mist1 protein affects digestive enzyme content. Examination of the ER and Golgi apparatus, which govern protein synthesis, revealed that the overall ER structure remained normal in Mist1$^{-/-}$ cells [29]. On the other hand, deletion of Mist1 modified the structure of the Golgi apparatus. Staining the Golgi with Man II revealed a diffused Golgi organization in Mist1$^{-/-}$ cells (Figure 8A). As expected, the diffused Golgi resulted in reduced digestive enzyme content in pancreatic acini. Mist1$^{-/-}$ cells contained 2.5-3 folds less amylase, trypsin and lipase than WT cells (Figure 8B). Amylase content measured in mice as young as 1 month and as old as 15 months were found to be similarly reduced, suggesting that reduction in digestive enzyme content does not develop
with time, but is a relatively early defect in Mist1−/− acinar cells. This observation is consistent
with Mist1 controlling the transcriptional regulation of genes involved in localization of several
organelles, including the secretory granules, the mitochondria and the Golgi apparatus in
pancreatic acini, possibly functioning as a master regulator of intracellular organelle localization.
Further studies will be needed to establish this intriguing possibility.

**Exocytosis and growth in Mist1−/− mice:** Changes in [Ca^{2+}]_{i} are the primary stimulator of
exocytosis in pancreatic acini [3] and aberrant Ca^{2+} signaling is intimately associated with
pancreatitis [6-8]. In addition, digestive enzymes content is low in Mist1−/− cells. Therefore, we
expected that digestive enzyme secretion would be modified in Mist1−/− cells and that the Mist1−/−
mice would show retarded growth or higher food consumption. Gross inspection of the
pancreases of Mist1−/− mice did not reveal any major differences in the size or shape of the organ.
However, the pancreas of Mist1−/− mice tended to be more fibrotic than that of WT mice, as
suggested by the need for longer digestion by collagenase to liberate Mist1−/− acini (not shown).
Large acinar clusters comprise of 8-30 cells prepared from WT and Mist1−/− mice were used to
measure stimulated amylase secretion as a measure of exocytosis. In the large clusters the
structure of the acini and stimulated enzyme secretion is preserved and faithfully reflects enzyme
secretion *in vivo* (41). As shown in Figure 9, exocytosis in response to both carbachol and CCK
was markedly impaired in Mist1−/− acini. In fact, no exocytosis could be measured in Mist1−/−
acini stimulated with physiological agonist concentrations. Low exocytosis in Mist1−/− acini was
observed only at very high agonist concentrations that are pathological in WT animals.
Furthermore, after correction for total amylase content, the peak amylase release by Mist1−/− acini
detected at the higher agonist concentrations was only 50% of that measured in WT acini. Since
high agonist concentrations increase [Ca^{2+}]_{i} to similar levels in WT and Mist1−/− cells (Figures 3,
4) this suggests that mislocalization of secretory granules contributed to the aberrant exocytosis.
This was tested directly by measuring the response to increasing [Ca^{2+}]_{i} with inomycin and
stimulating protein kinase C with PMA. Figure 9C shows that deletion of Mist1 impaired
exocytosis in response to both inomycin and PMA stimulation by about 40-50%. However, it is
important to note that exocytosis stimulated by physiological agonist concentrations was
impaired by 80-95%, indicating that impaired Ca^{2+} signaling was the major cause for the poor
exocytosis in Mist1−/− cells.
An important implication of the findings in Figure 9 is that the precise pattern of Ca\(^{2+}\) oscillations and waves are critical for exocytosis. Thus, CCK between 1-100 pM and carbachol at 0.5 and 1 µM evoked robust Ca\(^{2+}\) increases in Mist1\(^{-/-}\) cells, but did not stimulate any exocytosis. On the other hand, the same or lower agonist concentrations evoked a smaller Ca\(^{2+}\) signal but markedly stimulated exocytosis in WT acini. The only difference in the Ca\(^{2+}\) signals in WT and Mist1\(^{-/-}\) cells is that in WT cells the agonists induced repetitive Ca\(^{2+}\) oscillations and propagated Ca\(^{2+}\) waves.

The impaired stimulated exocytosis was expected to lead to malnutrition, retarded growth and/or increased food consumption in the Mist1\(^{-/-}\) mice. Remarkably, this was not the case for male or female Mist1\(^{-/-}\) mice (Figure 10A, 10B). Food consumption and growth were similar in WT and Mist1\(^{-/-}\) mice for the 32 weeks that the data were recorded. This was the case during both the rapid (first 10 weeks) and slow growth (weeks 11-32) phases of the animals development. These results suggest that either secretion \textit{in vivo} was affected less than observed with isolated acinar clusters or that the residual pancreatic function in Mist1\(^{-/-}\) mice is sufficient to support the food digestion and nutritional requirement of these animals.

In conclusion, the findings of the present work reveal several new roles for Mist1. We have extended previous studies to show that Mist1 controls the localization, and perhaps integrity, of the mitochondria and the Golgi apparatus, in addition to regulating the localization of secretory granules. The disordered localization of intracellular organelles leads to aberrant Ca\(^{2+}\) signaling that prevents Ca\(^{2+}\) oscillations and distorts the apical-to-basal Ca\(^{2+}\) waves. These findings provide evidence for the importance of cellular architecture in the generation and propagation of the Ca\(^{2+}\) signals. The aberrant Ca\(^{2+}\) signaling and cell architecture also results in greatly reduced exocytosis, further demonstrating the importance of Ca\(^{2+}\) signaling in exocrine pancreatic function. The unexpected finding is the lack of any effect of the compromised acinar pancreatic function on food consumption and animal growth. Whether this is the result of a less compromised pancreatic function \textit{in vivo} or the large spare capacity of the pancreatic function remains to be determined. However, given the known spare capacity of the pancreas and the fact that secretion by acinar clusters accurately reflects secretion \textit{in vivo} (41), it is likely that the spare pancreatic function protected the Mist1\(^{-/-}\) animals to allow normal growth with no need for increased food consumption.
References

1. Berridge MJ, Bootman MD, Roderick HL. (2003) Nat Rev Mol Cell Biol. 4:517-529.
2. Carafoli E. (2002) Proc Natl Acad Sci U S A. 99:1115-1122.
3. Williams JA. (2001) Ann Rev Physiol. 63:77-97.
4. Hankard R, Munck A, Navarro J. (2002) Horm Res. 58 (Suppl) 1:16-20.
5. Saluja AK, Bhagat L, Lee HS, Bhatia M, Frossard JL, Steer ML. (1999) Am J Physiol 276:G835-G842.
6. Kim JY, Kim KH, Lee JA, Namkung W, Sun AQ, Ananthanarayanan M, Suchy FJ, Shin DM, Muallem S, Lee MG. (2002) Gastroenterology 122:1941-1953.
7. Voronina S, Longbottom R, Sutton R, Petersen OH, Tepikin A. (2002) J Physiol. 2002; 540:49-55.
8. Mooren FCh, Hlouschek V, Finkes T, Turi S, Weber IA, Singh J, Domschke W, Schnekenburger J, Kruger B, Lerch MM. (2003) J Biol Chem. 278:9361-9369.
9. Kasai H, Li YX, Miyashita Y. (1993) Cell 74:669-677.
10. Thorn P, Lawrie AM, Smith PM, Gallacher DV, Petersen OH (1993) Cell 74:661-668.
11. Xu X, Zeng W, Diaz J, Muallem S. (1996) J Biol Chem. 271:24684-24690.
12. Straub SV, Giovannucci DR, Yule DI. (2000) J Gen Physiol. 116:547-560.
13. Kiselyov K, Shin DM and Muallem S. (2003) Cellular Signalling 15: 243-253.
14. Lee MG, Xu X, Zeng W, Diaz J, Wojcikiewicz RJ, Kuo TH, Wuytack F, Racymaekers L, Muallem S. (1997) J Biol Chem. 272:15765-15770.
15. Yule DI, Ernst SA, Ohnishi H, Wojcikiewicz RJ. (1997) J Biol Chem. 272:9093-9098.
16. Li Q, Luo X, Muallem S. (2004) J Biol Chem. 279:27837-27840.
17. Pozzan T, Magalhaes P, Rizzuto R. (2000) Cell Calcium. 28:279-283.
18. Tinel H, Cancela JM, Mogami H, Gerasimenko JV, Gerasimenko OV, Tepikin AV, Petersen OH. (1999) EMBO J. 18:4999-5008.
19. Park MK, Ashby MC, Erdemli G, Petersen OH, Tepikin AV. (2001) EMBO J. 20:1863-1874.
20. Bruce JI, Giovannucci DR, Blinder G, Shuttleworth TJ, Yule DI. (2004) J Biol Chem. 279:12909-12917.
21. Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, Pozzan T. (1998) Science. 280:1763-1766.
22. Rizzuto R, Brini M, Murgia M, Pozzan T. (1993) Science. 262:744-747.
23. Rizzuto R, Duchen MR, Pozzan T. Sci STKE. 2004; (215):re1.
24. Hoth M, Fanger CM, Lewis RS. (1997) J Cell Biol. 137:633-648.
25. Gilabert JA, Parekh AB. (2000) EMBO J. 19:6401-6407.
26. Pin CL, Rukstalis JM, Johnson C, Konieczny SF. (2001) J Cell Biol. 155:519-530.
27. Pin CL, Bonvissuto AC, Konieczny SF. (2000) Anat Rec. 259:157-167.
28. Rukstalis JM, Kowalik A, Zhu L, Lindington D, Pin CL, Konieczny SF. (2003) J Cell Sci.
116:3315-3325.
29. Zhu L, Tran T, Rukstalis JM, Sun P, Damsz B, Konieczny SF. (2004) Mol Cell Biol.
24:2673-2681.
30. Johnson CL, Kowalik AS, Rajakumar N, Pin CL. (2004) Mech Dev. 2004; 121:261-272.
31. Shin DM, Zhao XS, Zeng W, Mozhayeva M, Muallem S. (2000) J Cell Biol. 150:1101-1112.
32. Shin DM, Luo X, Wilkie TM, Miller LJ, Peck AB, Humphreys-Beher MG, Muallem S.
(2001) J Biol Chem. 276:44146-44156.
33. Luo X, Popov S, Bera AK, Wilkie TM, Muallem S. (2001) Mol Cell. 7:651-660.
34. Zhao XS, Shin DM, Liu LH, Shull GE, Muallem S. (2001) EMBO J. 20:2680-2689.
35. Gupta N, Rathi P, Gupta R. (2002) Anal Biochem. 311:98-99.
36. Gildberg A, Overbo K. (1990) Comp Biochem Physiol B. 97:775-7782.
37. Mercer LD, Beart PM. (2004) Neurosci Lett. 359:109-113.
38. Hattori M, Suzuki AZ, Higo T, Miyauchi H, Michikawa T, Nakamura T, Inoue T, Mikoshiba
K. (2004) J Biol Chem. 279:11967-11975.
39. Hajnoczky G, Robb-Gaspers LD, Seitz MB, Thomas AP. (1995) Cell. 82:415-424.
40. Jouaville LS, Pinton P, Bastianutto C, Rutter GA, Rizzuto R. (1999) Proc Natl Acad Sci.
96:13807-13812.
41. Williams JA, Korc M, Dormer RL (1978) Am J Physiol. 235:517-524.

Acknowledgement
This work was supported by NIH grants DK38938 and DE13902 (S.M.), grant No. R13-2003-13
from the Medical Science and Engineering Research Program of the Korea Science &
Engineering Foundation (D.M.S) and NIH grant DK55489 and the Purdue Cancer Center (S.F.K).
Figure 1: Expression of GPCRs in WT and Mist1\(^{-/-}\) cells.
CCK and M3 receptors and actin mRNA levels were evaluated by RT-PCR using RNA isolated from pancreatic acinar cells (A,) or brain (D) of WT and Mist1\(^{-/-}\) mice. Frozen pancreatic sections of WT (B) and Mist1\(^{-/-}\) mice (C) were used to immunolocalize the M3Rs. Brain extracts from 5 WT and 5 Mist1\(^{-/-}\) mice were used to analyze expression of M3Rs by Western blot.

Figure 2: IP\(_3\) production in WT and Mist1\(^{-/-}\) cells.
For measurement of IP\(_3\), pancreatic acini prepared from 2 months old WT and Mist1\(^{-/-}\) mice were stimulated with the indicated concentrations of CCK (A) or carbachol (B) for 5-10 seconds. IP\(_3\) was extracted and IP\(_3\) content in the extract was measured by a radioligand assay, as described in Methods.

Figure 3: CCK-evoked Ca\(^{2+}\) signaling in WT and Mist1\(^{-/-}\) cells.
WT (A) or Mist1\(^{-/-}\) cells (B) loaded with Fura2 were stimulated with the indicated concentrations of CCK. At the end of each experiment the cells were stimulated with 10 nM CCK to discharge all remaining ER Ca\(^{2+}\). The extent of stimulation at each CCK concentration was calculated from the reduction of the Ca\(^{2+}\) signal evoked by maximal CCK concentration to determine the dose response for CCK stimulation (C) for WT (■) and Mist1\(^{-/-}\) cells (●).

Figure 4: Carbachol-evoked Ca\(^{2+}\) signaling in WT and Mist1\(^{-/-}\) cells.
WT (A) or Mist1\(^{-/-}\) cells (B) were stimulated with the indicated carbachol concentrations. At the end of each experiment the cells were stimulated with 1 mM carbachol to calculate the extent of cells stimulation at each carbachol concentration and determine the dose response for Carbachol stimulation (C) for WT (■) and Mist1\(^{-/-}\) cells (●).

Figure 5: Ca\(^{2+}\) waves in Mist1\(^{+/-}\) and Mist1\(^{-/-}\) cells.
WT (A), Mist1\(^{+/-}\) (C) and Mist1\(^{-/-}\) cells (B, D) were stimulated with 10 pM CCK (A, B) or 5 µM carbachol (C, D) to better resolve the Ca\(^{2+}\) wave in Mist1\(^{-/-}\) cells. In each panel, the first image is the bright field image that also indicates the apical (green) and basal pole (red) areas analyzed.
The traces indicate the time course of the change in Fura2 fluorescence at the regions labeled in the bright field images. The fluorescence images depict the change in \([\text{Ca}^{2+}]_i\), at selective times during CCK or carbachol stimulation. The times in seconds at which the images were acquired are indicated by the yellow numbers.

**Figure 6: Expression of Ca\(^{2+}\) signaling proteins in WT and Mist1\(^{-/-}\) cells.**
Panel (A): Brain extracts from 5 WT and 5 Mist1\(^{-/-}\) mice were used to analyze expression of IP\(_3\)R3, IP\(_3\)R2, IP\(_3\)R1, SERCA2b and PMCA. Panel (B): Frozen pancreatic sections of WT and Mist1\(^{-/-}\) mice were used to immunolocalize IP\(_3\)R2 and IP\(_3\)R3, as indicated in the Figure.

**Figure 7: Mitochondrial and cytoplasmic Ca\(^{2+}\) in WT and Mist1\(^{-/-}\) cells.**
WT (A, B) and Mist1\(^{-/-}\) acini (C, D) were loaded with mitotracker green by a 10 min incubation with solution A containing 1 \(\mu\)M mitotracker green and imaged by confocal microscopy. Panels (A, C) are the bright field images and panels (B, D) are the corresponding fluorescence images. WT (E, G) and Mist1\(^{-/-}\) (F, H) acini were loaded with Fluo3 (green) and Rhod-2 (red) to measure cytosolic and mitochondrial Ca\(^{2+}\), respectively. The images show the Fluo3 and Rhod-2 fluorescence of resting cells. The cells were stimulated with 0.5 \(\mu\)M or 1 mM carbachol and the fluorescence changes in the cytosol (green traces) and mitochondria (red traces) were analyzed and plotted as arbitrary changes in fluorescence after normalization to the same scale. Similar results were obtained in 3 separate cell preparations with at least 10 acini. WT(I) and Mist1\(^{-/-}\) (J) acini were incubated with 1 \(\mu\)M TMRM for 5-7 min. The dye was then washed by perfusion and the cells exposed to 10 \(\mu\)M FCCP. The results show the mean±S.E.M. of 9-13 acini from two preparations.

**Figure 8: Localization of the Golgi apparatus and digestive enzyme content in WT and Mist1\(^{-/-}\) cells.**
Panel (A): Frozen pancreatic sections of WT and Mist1\(^{-/-}\) mice were used to immunolocalize Man II. Panel (B): Total extracts of pancreatic acini from WT and Mist1\(^{-/-}\) mice were used to measure amylase, trypsin and lipase activity. Digestive enzyme activities were calculated per mg protein and are expressed as the ratio of activities measured in WT and Mist1\(^{-/-}\) acini. The number of determinations from separate pancreases is indicated in each column.
Figure 9: Exocytosis in WT and Mist1−/− cells.

WT (■) and Mist1−/− (●) acini were stimulated for 30 min with the indicated concentrations of carbachol (A) or CCK (B). WT (open columns) and Mist1−/− acini (dark columns) were also stimulated with 10 μM carbachol, 5 μM inomycin (Ino) or 1 μM PMA for 5 or 30 minutes (C). Amylase activity in the supernatant was measured and calculated as % total activity in each group of acini. The results are the mean±S.E.M of 4 experiments in (A, B) and 3 experiments in (C).

Figure 10: Weight gain and food consumption of WT and Mist1−/− cells.

Groups of 8 WT male (■), WT female (□) Mist1−/− male (●) and Mist1−/− female (○) mice were kept with their mothers for 4 weeks. After 4 weeks mice from each group were housed in two cages. Weight was determined on the day of birth and for the subsequent 32 weeks daily or weekly, as needed. Measurement of food consumption started after weaning. Weight (A) was averaged each week and food consumption (B) per day was calculated from the weekly average. For the 16, 24 and 32 week time points weight gain and food consumption were determined only for the week of interest. None of the values for any given week were statistically different between WT and Mist1−/− mice.
Figure 1, Luo et al

**A) Pancreas**

|     | M | WT | Mist1<sup>-/-</sup> |
|-----|---|----|-------------------|
| 600 |   |     |                   |
| 500 |   |     |                   |
| 400 |   |     |                   |

CCKR

M3R

Actin

**B) M3R WT Pancreas**

**C) M3R Mist1<sup>-/-</sup> Pancreas**

**D) Brain, RT-PCR**

|     | M | WT | Mist1<sup>-/-</sup> |
|-----|---|----|-------------------|
|     |   |    |                   |
|     |   |    |                   |
|     |   |    |                   |

CCKR

M3R

Actin

**E) Brain, WB**

| WT | Mist1<sup>-/-</sup> | M |
|----|---------------------|---|
|    |                     | 175 kD |
Figure 2, Luo et al.

**A)**

IP$_3$ (pmole/mg protein) vs. CCK (M)

- WT
- Mist1$^{-/-}$

**B)**

IP$_3$ (pmole/mg protein) vs. Carbachol ($\mu$M)

- WT
- Mist1$^{-/-}$
Figure 3, Luo et al

**A) WT**

- **a)**
  - 1 pM CCK
  - 10 nM CCK
  - \(\Delta \text{Ca}^{2+}/500 \text{nM} \)
  - 5 min

- **b)**
  - 5 pM CCK
  - 10 nM CCK

- **c)**
  - 10 pM CCK
  - 10 nM CCK

- **d)**
  - 25 pM CCK
  - 10 nM CCK

- **e)**
  - 100 pM CCK
  - 10 nM CCK

**B) Mist1^-/-**

- **a)**
  - 1 pM CCK
  - 10 nM CCK

- **b)**
  - 5 pM CCK
  - 10 nM CCK

- **c)**
  - 10 pM CCK
  - 10 nM CCK

- **d)**
  - 25 pM CCK
  - 10 nM CCK

- **e)**
  - 100 pM CCK
  - 10 nM CCK

**C)**

- (1-residual 10 nM CCK)

- WT
- Mist1^-/-

CCK (pM)

(1-residual 10 nM CCK)
Figure 4, Luo et al

**A) WT**

- a) 0.0625 μM Carb 1 mM Carb
- b) 0.25 μM Carb 1 mM Carb
- c) 0.5 μM Carb 1 mM Carb
- d) 1 μM Carb 1 mM Carb
- e) 10 μM Carb 1 mM Carb

**B) Mist1−/−**

- a) 0.0625 μM Carb 1 mM Carb
- b) 0.25 μM Carb 1 mM Carb
- c) 0.5 μM Carb 1 mM Carb
- d) 1 μM Carb 1 mM Carb
- e) 10 μM Carb 1 mM Carb

**C)**

- WT
- Mist1−/−

![Graph](http://www.jbc.org/Downloaded from)

Carbachol (μM)
Figure 5, Luo et al

**A) Mist1**

- **Mist1**
- 10 pM CCK
- 1.0 0.0 0.2 0.4 0.6 0.8
- 1.4 1.8 2.2 2.6 3.0

**B) Mist1**

- **Mist1**
- 10 pM CCK
- 0.0 0.17 0.25 0.33 0.5
- 0.67 0.75 0.92 1.08 1.55

**C) Mist1**

- **Mist1**
- 5 μM Carbachol
- 0.0 0.03 0.09 0.21 0.33
- 0.39 0.51 0.63 0.81 1.11

**D) Mist1**

- **Mist1**
- 5 μM Carbachol
- 0.0 0.06 0.12 0.18 0.24
- 0.36 0.42 0.48 0.54 0.9
Figure 6, Luo et al

A) WB

|        | WT          | Mist1−/−   |
|--------|-------------|------------|
| IP₃R3  |             |            |
| IP₃R2  |             |            |
| IP₃R1  |             |            |
| SERCA2b|             |            |
| PMCA   |             |            |

B) Localization of IP3Rs

[Images showing WB bands and localization microscopy for WT and Mist1−/− mice with IP₃R2 and IP₃R3]
Figure 7, Luo et al.
A) Localization Man II

B) Digestive enzyme content

| Enzymes content (WT/Mist1⁻/⁻) | Amylase | Trypsin | Lipase |
|--------------------------------|---------|---------|--------|
|                                | 9       | 4       | 3      |
Figure 9, Luo et al
Figure 10, Luo et al

A Weight gain

- **WT, Male**
- **Mist1−/−, Male**
- **WT, Female**
- **Mist1−/−, Female**

B Food consumption

- **WT, Male**
- **Mist1−/−, Male**
- **WT, Female**
- **Mist1−/−, Female**
Aberrant localization of intracellular organelles, Ca2+ signaling and exocytosis in Mist1 null mice
Xiang Luo, Dong Min Shin, Xinhua Wang, Stephen F. Konieczny and Shmuel Muallem

J. Biol. Chem. published online January 21, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M411973200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts