Aberrant Localization of Intracellular Organelles, Ca$^{2+}$ Signaling, and Exocytosis in Mist1 Null Mice*

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Ca$^{2+}$ 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 basic helix-loop-helix transcription factor. Ca$^{2+}$ signaling and amylase release were examined in pancreatic acini of wild type and Mist1 null mice to gain insight into the importance of cellular architecture for Ca$^{2+}$ signaling and regulated exocytosis. Mist1$^{-/}$ acinar cells exhibited dramatically altered Ca$^{2+}$ signaling with up-regulation of the cholecystokinin receptor but minimal effect upon expression of the M3 receptor. However, stimulation of inositol 1,4,5-trisphosphate production by cholecystokinin and carbachol was inefficient in Mist1$^{-/}$ cells. Although agonist stimulation of Mist1$^{-/}$ cells evoked a Ca$^{2+}$ signal, often the Ca$^{2+}$ increase was not in the form of typical Ca$^{2+}$ oscillations but rather in the form of a peak/plateau-type response. Mist1$^{-/}$ cells also displayed distorted apical-to-basal Ca$^{2+}$ waves. The aberrant Ca$^{2+}$ signaling was associated with mislocalization and reduced Ca$^{2+}$ uptake by the mitochondria of stimulated Mist1$^{-/}$ cells. Deletion of Mist1 also led to mislocalization of the Golgi apparatus and markedly reduced digestive enzyme content. The combination of aberrant Ca$^{2+}$ 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 Ca$^{2+}$ 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.

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)$^1$ 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 the 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 inositol 1,4,5-trisphosphate (IP$_3$) as well as the biophysical component that includes the Ca$^{2+}$ release and influx channels and the sarco/endoplasmic reticulum ATPase (SERCA2b) and plasma membrane Ca$^{2+}$ ATPase (PMCA) pumps (16).

Other important regulators 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, Pozzan and co-workers 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 $I_{	ext{vac}}$ 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, namely 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, a ring surrounding the nucleus, and a string lining the plasma membrane (19).

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1 The abbreviations used are: GPCR, G proteins coupled receptor; CCK, cholecystokinin; CCKR, cholecystokinin; ER, endoplasmic reticulum; IP$_3$, inositol 1,4,5-trisphosphate; IP$_3$R, IP$_3$ receptor; M3R, M3 muscarinic receptor; PMCA, plasma membrane Ca$^{2+}$ ATPase pump; RT, reverse transcription; SERCA2b, sarco/endoplasmic reticulum Ca$^{2+}$ ATPase pump; TMRM, tetramethylrhodamine methyl ester; WT, wild type.
Ca\textsuperscript{2+} Signaling and Exocytosis in Mist1\textsuperscript{-/-} Cells

The intricate localization of Ca\textsuperscript{2+} signaling complexes and intracellular organelles is likely to be critical for the precise operation of the Ca\textsuperscript{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 Ca\textsuperscript{2+} response and exocytosis in acinar cells. In the present work we show that the Ca\textsuperscript{2+} signaling machinery in Mist1\textsuperscript{-/-} cells is dramatically altered, resulting in aberrant agonist-evoked Ca\textsuperscript{2+} oscillations and Ca\textsuperscript{2+} waves. The altered Ca\textsuperscript{2+} response is not due to mislocalization of the remaining IP\textsubscript{3} receptors but rather is a consequence of mislocalization and aberrant Ca\textsuperscript{2+} uptake into mitochondria. The Golgi apparatus of Mist1\textsuperscript{-/-} cells is diffuse and fragmented, which may explain the markedly decreased digestive enzyme content in the secretory granules. Exocytosis by Mist1\textsuperscript{-/-} 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\textsuperscript{-/-} mice. These findings provide experimental evidence for the importance of cellular architecture in the generation and propagation of Ca\textsuperscript{2+} signals and illustrate the sparse capacity of the exocrine pancreas to allow normal growth at reduced exocytosis.

**EXPERIMENTAL PROCEDURES**

**Materials**—Carbachol, CCK, and phorbol 12-myristate 13-acetate were purchased from Sigma. IP\textsubscript{3} was from Alexis. Fura2/AM, Mito-Tracker Green, Rhod-2, and Flu-3 were from TefLabs. Tetramethylrhodamine methyl ester (TMRM) was from Molecular Probes. Dr. Akihiko Tanimura (University of Hokkaido, Japan) generously provided anti-IP\textsubscript{3}R1, IP\textsubscript{3}R2, and IP\textsubscript{3}R3 polyclonal antibodies. Anti-PMCA mAb 5F10 was purchased from Affinity Bioreagents. Anti-mannosidase II polyclonal antibodies were obtained from the University of Georgia. The polyclonal antibody against SERCA2b was provided by Dr. Frank Wuytack (University of Leuven, Belgium). Anti-M3 receptors antibodies were from US Biological.

**Experimental Animals, Body Weights, and Food Intake—**Mist1\textsuperscript{-/-} 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 conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. WT and Mist1\textsuperscript{-/-} mice were housed individually. Food intake was measured three times per week using an Ohaus portable electronic scale with a sensitivity of 0.1 g. The body weight of litter mates was measured on the day of birth and then at 1-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 (140 mM NaCl, 5 mM KCl, 1 mM MgCl\textsubscript{2}, 1 mM CaCl\textsubscript{2}, 10 mM HEPES (pH 7.4 with NaOH), 10 mM glucose, 0.1% bovine serum albumin, and 0.02% soybean trypsin inhibitor) and kept on ice until used.

**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 from the acinar cells and brains of WT and Mist1\textsuperscript{-/-} mice with TRIzol reagent (Invitrogen) and dissolved in diethyl pyrocarbonate-treated water. RT-PCR was performed using the same amount of RNA isolated from three WT and three Mist1\textsuperscript{-/-} mice. The RT reaction was performed with the SuperScript\textsuperscript{TM} II RT kit (Invitrogen) in a 20-\muL reaction volume as suggested by the manufacturer. PCR primers were designed using Primer 3 version 0.2.1 and alignment by NCBi BLAST software. The primer sequences used were as follows: CCK receptor sense, 5’-TCAGTGGCATCTCATGGTTC3’- and CCK receptor antisense, 5’-ATGAGTCGATGAAACCCAC3’- (size of PCR product, 442 bp); muscarinic receptor 3 sense, 5’-TGTTGGTGATACAGCTGTG3’- and muscarinic receptor 3 antisense, 5’-TGGTGCTGTTGTTGGTGG3’- (size of PCR product, 427 bp); \&-actin sense, 5’-GTTTCCAACTGGCAGCA3’- and \&-actin antisense, 5’-TCTGAGCTGTGGTGAAG3’- (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 s of incubation at 94 °C, 1 min at 55 °C, and 1 min at 72 °C, extended by 10 min at 72 °C, and terminated by a 10-min incubation at 4 °C. Preliminary experiments showed that 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\textsuperscript{-/-} mice in a buffer containing (pH 7.6 with KOH) 100 mM KCl, 20 mM Tris-base, 1 mM EDTA, 1 mM benzamidine, and 1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 1000 × g for 10 min at 4 °C. The supernatant was
reduce variation due to feeding schedule, the mice were fasted for 24 h before the preparation of acini, and the results are expressed as the ratio between the activities in WT/Mist1−/− cells. For the 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 of incubation at 37 °C, samples were transferred to Eppendorf tubes, the supernatants were separated from the acini by centrifugation, and the 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 Phadebas kit (Pharmacia and Upjohn Diagnostics, catalog number 10-5380-33) as described previously (34). In brief, 10-μl samples were diluted into 200 μl of buffer containing 20 mM NaH2PO4, 20 mM Na2HPO4, 50 mM NaCl, and 0.02% NaN3, pH 7.0. 20 μl of the diluted samples were mixed with 1 ml of blue starch (10 mg/ml) and incubated for 10 min at 37 °C with gentle shaking. The reaction was stopped by the addition of 250 μl of 2 M NaOH, the supernatant was cleared by 5 min of centrifugation at 14,000 rpm, and the absorbance was measured at a wavelength of 595 nm.

Lipase activity was measured using para-nitrophenyl palmitate as a substrate (35). Samples of 0.1 ml were added to 2.4 ml of a freshly prepared para-nitrophenyl palmitate solution (30 mg of para-nitrophenyl palmitate in 100 ml of 100 mM Tris buffer, pH 8.5, 207 mg of Na+-deoxycholate, and 100 mg of gum arabic) and incubated for 1 h at 37 °C with gentle shaking. The reactions were terminated by the addition of 0.2 ml of 100 mM CaCl2, the samples were 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-α-benzoyl-arginine-p-nitroanilide as described previously (36) with a slight modification. Samples of 100 μl were added into 2.4 ml of 1 mM substrate N-α-benzoyl-arginine-p-nitroanilide dissolved in a solution containing 100 mM Tris buffer, pH 9.0, and 10 mM CaCl2, and incubated for 1 h at 37 °C with gentle shaking. 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. of the indicated number of experiments. Statistical significance was evaluated by a two-way analysis of variance. All immunostaining experiments were repeated at least five times with similar results.

RESULTS AND DISCUSSION

Impaired Stimulation of Ca2+ Signaling in Mist1−/− Cells—The up-regulation of the CCK receptor mRNA in Mist1−/− mouse pancreatic acinar cells (26) was confirmed by RT-PCR in pancreatic acinar cells (Fig. 1A). However, this increase was not observed for all GPCRs. Initial analysis by RT-PCR showed that expression of M3R mRNA in the pancreas of Mist1−/− mice was unchanged or slightly up-regulated (Fig. 1A). An attempt to quantitate the extent of protein expression of M3Rs...
The aberrant localization of secretory granules, the reduction in expression of IP$_3$R3, and the up-regulation of CCK receptor 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 the Mist1 protein reduced the EC$_{50}$ for CCK stimulation from ~0.83 to 0.032 nM and reduced the maximal production of IP$_3$ by ~50% (Fig. 2A). Mist1$^{-/-}$ acinar cells showed an increased EC$_{50}$ for carbachol from ~3.7 to ~46 nM and a reduced maximal production of IP$_3$ of ~30% (Fig. 2B). The increased apparent affinity to CCK may relate to the increased mRNA levels of the CCK receptors (26) (Fig. 1A). The modest change in M3R mRNA and protein in the brains of the mice is consistent with this interpretation. However, the reduction in maximal IP$_3$ production indicates a generally impaired signaling by Mist1$^{-/-}$ cells. Measurement of IP$_3$ production showed that signaling by CCK may relate to the increased mRNA levels of the CCK receptors (26) (Fig. 1B). The modest change in M3R mRNA and protein reduced the EC$_{50}$ for CCK stimulation from 0.032 nM to ~0.063 nM and reduced the maximal production of IP$_3$ by ~30%. However, the antibodies gave a reasonable signal in immunostaining. Fig. 1A, show enrichment of M3R protein in the brains of the mice. Fig. 1B, show enrichment of M3R mRNA and protein in the brains of Mist1$^{-/-}$ mice. Western blot findings in the pancreas, brain mRNA for CCK receptors was reduced, and for M3Rs it was unchanged. Western blot analysis of extracts prepared from five brains of WT and five brains of Mist1$^{-/-}$ mice showed a small reduction in the level of M3R protein in Mist1$^{-/-}$ cells (18 ± 5%, n = 5) (Fig. 1E).

The consequence of impaired IP$_3$ production on the pattern of Ca$^{2+}$ signaling is shown in Figs. 3 and 4. Panels A and B in each figure show example traces from individual experiments, whereas the panels C (Figs. 3 and 4) summarize results from multiple experiments. CCK stimulated Ca$^{2+}$ signaling with a higher affinity in Mist1$^{-/-}$ cells (Fig. 3C), whereas the affinity for carbachol to trigger a Ca$^{2+}$ signal was lower in Mist1$^{-/-}$ cells (Fig. 4C), as was expected from their effects on IP$_3$ production. However, maximal concentrations of both agonists increased [Ca$^{2+}$], to similar levels in WT and Mist1$^{-/-}$ cells (CCK, 723 ± 81 nM in WT and 744 ± 79 nM in Mist1$^{-/-}$; carbachol, 726 ± 88 nM in WT and 711 ± 63 nM in Mist1$^{-/-}$; n = 6–7 acini from 6–7 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$^{2+}$] oscillations in the Mist1$^{-/-}$ cells but rather a single Ca$^{2+}$ transient with a subsequent plateau (Figs. 3, A and B, and 4, A and 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 µM carbachol (not shown) or 10 µM CCK (Fig. 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, Fig. 5B). However, we noted that [Ca$^{2+}$], 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 µM carbachol generated Ca$^{2+}$ waves that propagated through the cell at a rate of 16.7 ± 0.5 µm/sec (Fig. 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 (Fig. 5B). First, the Ca$^{2+}$ wave...
in Mist1<sup>−/−</sup> cells propagated very rapidly. When it could be resolved, the Ca<sup>2+</sup> wave propagated at a rate of 43.6 ± 5.8 (n = 14) μm/s. Second, the Ca<sup>2+</sup> wave in Mist1<sup>−/−</sup> cells propagated along the cell periphery and only then proceeded concentrically toward the cell center (Fig. 5D). These results suggest that the proteins and organelles that control the propagation of the Ca<sup>2+</sup> wave do not function properly in the Mist1<sup>−/−</sup> cells. This can be due to mislocalization of IP<sub>3</sub>R<sub>s</sub> that are concentrated at the apical pole of pancreatic acini (14, 15), mislocalization of the mitochondria that determine the pattern of the Ca<sup>2+</sup> wave (18–20), or both.

**Expression and Localization of Ca<sup>2+</sup> Signaling Proteins**—The Ca<sup>2+</sup> waves in pancreatic acini are regulated by localization of IP<sub>3</sub> receptors (14, 15) and other Ca<sup>2+</sup> signaling proteins (13, 16) and by Ca<sup>2+</sup> uptake into the mitochondria (18–20). Our previous work has shown that the level of IP<sub>3</sub>R3 is down-regulated in Mist1<sup>−/−</sup> cells (26). To test if the down-regulation of IP<sub>3</sub>R3 is a more general phenomenon and whether deletion of the Mist1 gene affects localization of Ca<sup>2+</sup> signaling complexes, we analyzed the expression of individual IP<sub>3</sub>Rs in brain extracts and the localization of IP<sub>3</sub>R2 and IP<sub>3</sub>R3 in pancreatic acini. The Western blot analysis in Fig. 6A shows that deletion of Mist1 reduced expression of IP<sub>3</sub>R3 in the brain by 65 ± 11% (n = 5). On the other hand, expression of IP<sub>3</sub>R1, IP<sub>3</sub>R2, SERCA2b, and PMCA was not affected. This indicates that down-regulation of IP<sub>3</sub>R3 expression in Mist1<sup>−/−</sup> mice is not specific to acinar cells, raising the possibility that loss of Mist1 may effect IP<sub>3</sub>R3 expression in other cell types. The immunolocalization in Fig. 6B revealed a lack of IP<sub>3</sub>R3 and completely normal localization of IP<sub>3</sub>R2 in pancreatic acini of Mist1<sup>−/−</sup> cells.

The results in Fig. 6 indicate that the distorted Ca<sup>2+</sup> signaling and Ca<sup>2+</sup> waves observed in Mist1<sup>−/−</sup> cells are not due to a general aberrant expression of Ca<sup>2+</sup> signaling proteins or to mislocalization of the remaining IP<sub>3</sub>Rs. Furthermore these findings and those in Figs. 3 and 4 indicate that the function of IP<sub>3</sub>R3, and perhaps other IP<sub>3</sub>Rs, is cell-specific. A recent work provides strong evidence in cell lines that IP<sub>3</sub>R1 is essential for receptor-triggered Ca<sup>2+</sup> oscillations, whereas IP<sub>3</sub>R3 functions as an anti-Ca<sup>2+</sup> oscillatory unit (38). That is, knock-down of IP<sub>3</sub>R3 by small interfering RNA enhanced Ca<sup>2+</sup> oscillations. On the other hand, Ca<sup>2+</sup> oscillations were rare in Mist1<sup>−/−</sup> cells, where the IP<sub>3</sub>R3 is markedly down-regulated (Figs. 3 and 4). This would suggest a cell-specific function of IP<sub>3</sub>R3.

**Mitochondrial Localization and Function in Mist1<sup>−/−</sup> Cells**—The Ca<sup>2+</sup> waves in pancreatic acini and Ca<sup>2+</sup> signaling in many cells are prominently regulated by Ca<sup>2+</sup> uptake into the mitochondria (18–25). Therefore, we next compared localization and function of the mitochondria in WT and Mist1<sup>−/−</sup> cells. Localization of mitochondria was followed using MitoTracker Green detection, and mitochondrial and cytosolic Ca<sup>2+</sup> levels were measured with Rhod-2 and Fluo-3, respectively. As expected, the energized mitochondria in WT cells were clustered around the secretory granules and the nucleus and next to the plasma membrane, but they were completely excluded from the secretory granule area in pancreatic acini (Fig. 7, A, B, and E). By contrast, mitochondria in Mist1<sup>−/−</sup> acinar cells were highly disorganized (Fig. 7, C, D, and F). Energized mitochondria were found at all regions of the basal pole. In addition, although mostly excluded from the apical pole in Mist1<sup>−/−</sup> 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<sup>−/−</sup> cells resulted in the inhibition of Ca<sup>2+</sup> uptake into the mitochondria when the cells were stimulated with low agonist concentrations. In WT cells, [Ca<sup>2+</sup>], and mitochondrial Ca<sup>2+</sup> increases...
Mitochondrial Ca\(^{2+}\) signaling plays an important role in controlling the Ca\(^{2+}\) uptake into the mitochondria at a low agonist concentration. When the cells are stimulated at a low agonist concentration, such an uptake requires close communication between the mitochondria and the ER for Ca\(^{2+}\) entry. Previous work has emphasized the importance of the close apposition of the mitochondria to the ER for Ca\(^{2+}\) uptake into the mitochondria at intermediate agonist concentrations. Mitochondrial localization has a critical role in Ca\(^{2+}\) signaling. Mitochondria play a major role in mitochondrial and cellular energy metabolism and the regulation of genes involved in the localization of several organelles, including the secretory granules, the mitochondria, and the Golgi apparatus in pancreatic acini. Mitochondrial localization has a critical role in Ca\(^{2+}\) signaling.

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 the Mist1 protein affects digestive enzyme content. Examination of the ER and the 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 mannosidase II revealed a diffused Golgi organization in Mist1\(^{-/-}\) cells (Fig. 8A). As expected, the diffused Golgi resulted in reduced digestive enzyme content in pancreatic acini. Mist1\(^{-/-}\) cells contained 2.5–3-fold less amylase, trypsin, and lipase than WT cells (Fig. 8B). Amylase content measured in mice as young as 1 month and as old as 15 months was 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 the localization of several organelles, including the secretory granules, the mitochondria, and the Golgi apparatus in pancreatic acini, and possibly functioning as a master regulator of intracellular organelle localization. Further studies will be needed to establish this intriguing possibility.

![Figure 6](image1.png)

**Fig. 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 IP3R3, IP3R2, IP3R1, SERCA2b, and PMCA. Panel B, frozen pancreatic sections of WT and Mist1\(^{-/-}\) mice were used to immunolocalize IP3R2 and IP3R3 as indicated.

![Figure 7](image2.png)

**Fig. 7.** Mitochondrial and cytoplasmic Ca\(^{2+}\) in WT and Mist1\(^{-/-}\) cells. WT (A and B) and Mist1\(^{-/-}\) acini (C and 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 and C are the bright field images, and panels B and D are the corresponding fluorescence images. WT (E and G) and Mist1\(^{-/-}\) (F and 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 three separate cell preparations with at least 10 acini. WT (D) 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 were exposed to 10 \(\mu\)M carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone (FCCP). The results show the mean ± S.E. of 9–13 acini from two preparations.
Exocytosis and Growth in Mist1−/− Mice—Changes in [Ca^{2+}], are the primary stimulators of exocytosis in pancreatic acini (3), and aberrant Ca^{2+} signaling is intimately associated with pancreatitis (6–8). In addition, digestive enzyme 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 the stimulated enzyme secretion is preserved and faithfully reflects enzyme secretion in vivo (41). As shown in Fig. 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 the 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. Because high agonist concentrations increase [Ca^{2+}], to similar levels in WT and Mist1−/− cells (Figs. 3 and 4), this finding suggests that mislocalization of secretory granules contributed to the aberrant exocytosis. This was tested directly by measuring the response to increasing [Ca^{2+}], with ionomycin and stimulating protein kinase C with phorbol 12-myristate 13-acetate. Fig. 9C shows that deletion of Mist1 impaired exocytosis in response to both ionomycin and phorbol 12-myristate 13-acetate stimulation by ~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 Fig. 9 is that the precise pattern of Ca^{2+} oscillations and waves are critical for exocytosis. Thus, CCK between 1 and 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 (Fig. 10, A and B). 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 in vivo was affected less than what was 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 the 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 Ca2+ signaling that prevents Ca2+ oscillations and distorts the apical-to-basal Ca2+ waves. These findings provide evidence for the importance of cellular architecture in the generation and propagation of the Ca2+ signals. The aberrant Ca2+ signaling and cell architecture also result in greatly reduced exocytosis, further demonstrating the importance of Ca2+ 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 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 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

37. Deleted in proof
Aberrant Localization of Intracellular Organelles, Ca\(^{2+}\) Signaling, and Exocytosis in Mist1 Null Mice

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