Probing β-Cell Biology in Space and Time

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β-Cells in the islet of Langerhans have a central role in maintaining energy homeostasis. Understanding the physiology of β-cells and other islet cells requires a deep understanding of their structural and functional organization, their interaction with vessels and nerves, the layout of paracrine interactions, and the relationship between subcellular compartments and protein complexes inside each cell. These elements are not static; they are dynamic and exert their biological actions at different scales of time. Therefore, scientists must be able to investigate (and visualize) short- and long-lived events within the pancreas and β-cells. Current technological advances in microscopy are able to bridge multiple spatiotemporal scales in biology to reveal the complexity and heterogeneity of β-cell biology. Here, I briefly discuss the historical discoveries that leveraged microscopes to establish the basis of β-cell anatomy and structure, the current imaging platforms that allow the study of islet and β-cell biology at multiple scales of resolution, and their challenges and implications. Lastly, I outline how the remarkable longevity of structural elements at different scales in biology, from molecules to cells to multicellular structures, could represent a previously unrecognized organizational pattern in developing and adult β-cells and pancreas biology.

The human life span can be very long. The median life expectancy across the globe is between 70 and 75 years of age, and the number of people 80 years of age or older is projected to double in the next three decades (1). Therefore, a better understanding of the human longevity and aging processes is crucial to support present and future socioeconomic and health care needs of the human race. This remarkable longevity implies that human organs, cells, and intracellular components must have the resiliency to potentially endure decades of exposure to genetic and/or environmental stressors while sustaining a functioning human body. This raises a number of exciting fundamental questions: How do you build and maintain a whole organ for >80 years? What are the strategies that largely postmitotic cells use to maintain structure-function for decades? Are they similar across different types of organs? What is the impact of decades of exposure to by-products of metabolism on cells and tissues?

Aging associates with declines in cell function and/or metabolism in several organs, which align with declines in cognitive, cardiovascular, metabolic, and muscular functions, as well as a higher risk of developing metabolic diseases like type 2 diabetes (T2D) (2). T2D is caused by the collapse of normal glucose homeostasis mechanisms, which leads to significant complications that increase the risk of death (3). Nearly 840 million people worldwide are at risk for developing or have T2D, a disease that poses an immense burden on individuals and health care infrastructures (3). T2D incidence depends on sex and age and is generally characterized by a decline in β-cell function and insulin release (2,4); however, more recent studies have revealed the equally important role of non-β cells and the islet vasculature and nerves in regulating β-cell function in T2D pathophysiology (5–8).

The complex architecture of the pancreas, where rare clusters of endocrine cells called islets of Langerhans are surrounded by exocrine cells, poses a significant challenge to the visualization of islet cell structure, mass, and function. Today, islet scientists have access to a comprehensive toolbox of technologies that allow the interrogation of islet cell and protein function with remarkably high spatial and/or temporal resolution. The almost daily output of data from such techniques reveals the heterogeneous and dynamic nature of islet cell identity, structure, and functional parameters. Modern imaging approaches carry the potential to reveal how homeostatic processes important for maintenance of β-cell structure-function are affected by diabetes and to quantify the impact of different antidiabetes treatments on β-cell physiology.

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The main goal of this perspective is to briefly review the application (and challenges) of microscopy techniques to investigate the physiology of islet cells at different scales of resolution in space and time. Lastly, I would like to propose a basic organization pattern dependent on the remarkable longevity of cells and macromolecules, which may be important for islet cell development and function.

A Brief History of Cellular Homeostasis and Turnover

The development and maintenance of adult tissue architecture requires the precise coordination of signaling mechanisms that take place in each cell and across cell populations. Every day, cells are under pressure to maintain normal gene transcriptional programs, to translate millions of mRNA molecules into peptide sequences that must be transported, folded, and/or degraded correctly and in a timely fashion. Nutrients must be metabolized properly to meet energetic and biosynthetic demands of the cell. Dysregulation of many of these cell homeostasis pathways underlies the loss of cell identity and/or function in different tissues, including the pancreas, and are associated with aging, cancer, T2D, and other metabolic and degenerative diseases. Therefore, understanding how basic homeostatic mechanisms sustain cell and tissue structure-function over short and long periods of time, and how these aspects are affected by disease, is a fundamental problem in human biology.

Strikingly, the notion that cellular structures turn over is relatively new. Prior to the 1940s, scientists believed that tissue structures, such as cells and proteins, were static over time and suffered "wear and tear" as the organism aged. This notion began to radically change after the discovery of stable isotopes with a strong biological relevance, such as nitrogen-15 (^{15}N) (9). Stable isotopes allowed scientists to follow biological reactions for the first time in living organisms without the caveat of radioactive decay. These efforts were pioneered by pulse-chase experiments in which rats were fed a diet enriched with ^{15}N-labeled amino-acid leucine (^{15}N-Leu) or glycine (^{15}N-Gly) for 3 days. After this pulse-labeling period, scientists quantified the amount of ^{15}N-Leu and ^{15}N-Gly present in the protein fractions of several organs. Contrary to what was expected, they observed that most ^{15}N was retained in somatic organs, including the liver, muscle, and kidney (10). These findings demonstrated for the first time the dynamics of organ homeostasis and nutrient metabolism and led to the conclusion that "all constituents of living matter, whether functional or structural, of simple or complex constitution, are in a steady state of rapid flux." These findings were quickly followed by quantification of carbon-14 (^{14}C) integrated in biological structures to estimate the age of organisms (11), including cells in autoradiographs (12). More recently, scientists took advantage of large amounts of ^{14}C deposited in the Earth's atmosphere by ground nuclear tests in the 1950s, which virtually "pulse-labeled" all beings born in that decade, to determine the longevity of cells in humans (13), including β-cells (14). Together, these landmark discoveries established the concept of "metabolic regeneration" we know today and created a new field in chemistry and biology where scientists could investigate the metabolism, aging, and longevity of any cell in any organism across short and long time scales.

However, these approaches lack the spatial resolution required to establish the exact location of short and long-lived structures in tissues, cells, and protein complexes without disrupting their natural tissue microenvironment. Therefore, methods to visualize and quantify cell and molecular function and turnover in space and time are essential to enhance our current understanding of cell biology.

Microscopy Platforms in the Islet Cell Biology Field

The invention of light-based microscopes in the 17th century gave birth to the field of cell biology and to the realization that organisms can be made of trillions of individual and different types of cells. Using a microscope, Paul Langerhans jump-started the islet research field when he visualized "... small cells of almost perfect homogeneous content and of a polygonal form, with round nuclei without nucleoli, mostly lying together in pairs or small groups" (15). This discovery laid the foundation of modern islet research, launching scientists around the globe on a quest to understand islet cell structure-function and diabetes pathophysiology that continues to this day.

β-Cell Structure-Function and Heterogeneity Revealed by Multimodal Microscopy

Modern applications of light and electron microscopes allow the visualization of dynamic cellular events with high spatial and temporal resolution, from gene transcription and chromatin ultrastructure to organelles and cells and to organs and whole organisms. The islet field has benefited from these technological advancements, many of which are now part of the basic arsenal used to interrogate the molecular phenotype and structure-function of most (if not all) cell types in the islet microenvironment. For example, pioneering experiments of rat β-cells loaded with the calcium indicator Fura2 revealed differences in the response of individual β-cells to nutrient stimulation (16). This study allowed the appreciation of how β-cell functional heterogeneity is influenced by changes in glucose concentrations, which remains an area of high interest and that is now fueled by the application of advanced single-cell imaging and transcriptomics platforms (reviewed in ref. 17). In addition, application of light microscopy has led to descriptions of species-specific patterns in islet cytoarchitecture (18), innervation (19), and vascularization (20).

These concepts were expanded to larger tissue volumes using tissue-clearing technologies imaged using optical projection tomography (21) and light sheet microscopy (22). Recent technological developments allow acquisition

of the molecular signatures of cells in a multiplexed and spatially resolved manner in tissue sections or isolated cells. These imaging platforms (e.g., CODEX [23], imaging mass cytometry [24]) were initially developed in the cancer and immunology fields and rely on sequential rounds of staining and/or detection of multiple antibodies per sample. This creates multiplexed two-dimensional molecular maps of tissue architecture containing millions of cells preserved in their natural environment with single-cell resolution (usual resolution range is between 0.2 and 1.0 μm depending on the imaging platform used). This multiplexed information allows the identification of cell signatures and cell-cell associations within a specific tissue neighborhood or compartment. In the pancreas field, imaging mass cytometry has been successfully used to characterize markers of β-cell heterogeneity and the different types of immune cells present in the nondiabetic and type 1 diabetes (T1D) pancreas (25). In addition, the combinatorial expression of two β-cell surface markers (i.e., CD99 and ST8SIA1) was used to identify four transcriptionally and functionally distinct types of β-cells (26), while single-molecule fluorescence in situ hybridization (smFISH) experiments identified distinct β-cell types based on mRNA levels of insulin and the transcription factor Nkx2.2 (27). More recently, same-cell electrophysiology followed by single-cell mRNA sequencing (called patch-seq) was used to determine the correlation between β-cell gene transcription patterns and functional parameters, including exocytosis and Ca^{2+} and Na^{2+} ion dynamics (28). Together, these studies highlight the potential of correlated microscopy, electrophysiology, and sequencing applications to characterize the molecular and spatial characteristics that underlie the heterogeneity of β-cells within the complex architecture of the islet.

Historically, β-cell function has been largely studied using isolated islets. However, this requires digestion of the tissue, which damages the islet ultrastructure, possibly triggering inflammatory processes that impact cell activity and viability (29). As an alternative, the preparation of pancreatic slices has emerged as a new platform to study mouse and human islet cell function (30). Cells in slices can remain alive with largely preserved cell function, architecture, viability, and expression of genetically encoded calcium indicators for several days. These characteristics enabled visualization of β-cell intracellular calcium dynamics ($[Ca^{2+}]_i$) and network connectivity (31), islet macrophage physiology (32), and pericyte-mediated control of the islet vasculature (33) and revealed dysfunctional β-cell activity profiles in human T2D pancreas biopsies (34). In the future, this approach will likely benefit from addition of low-volume microfluidic perfusion systems, electrophysiological measurements, and/or spatial mRNA sequencing pipelines. Imaging β-cells in deeper layers of the pancreatic slices remains challenging due to resolution constraints of more commonly used light-based microscopes; however, this issue is likely to be resolved in the near future by the application of light sheet microscopes to perform high-speed imaging of fluorescent markers hundreds of microns deep into the tissue.

In Vivo Imaging Methods Shed Light on β-Cell Structure-Function

The human islet is composed of molecular and functionally distinct β-cell populations (26,35). To study their properties in vivo, one must be able to visualize them with high spatiotemporal resolution in vivo and in three dimensions. To achieve this, there are currently two major techniques available: In the first, a surgically implanted "body window" is positioned against the pancreas in the abdominal cavity of mice that are placed on top of an inverted light microscope. This technique allows the user to determine relative changes in islet β-cell mass, islet vascularization, and blood flow patterns (36), as well as reactive oxygen species and $[Ca^{2+}]_i$ levels (37). In the second, isolated islets are transplanted into the anterior chamber of the eye, where they engraft and regulate the host glucose homeostasis (38). This technique allows fast and longitudinal imaging of islet mass and functional parameters throughout the lifetime of the graft and/or the animal. When coupled with molecular reporters of cell identity, function, and/or viability, this technique revealed cellular events involved in islet allograft rejection (39), changes in β-cell NADPH (40) and $[Ca^{2+}]_i$ levels (41), the existence of functionally distinct β-cell types (42), quantified changes in β-cell mass due to β-cell proliferation (43), changes in the islet blood flow pattern (6,44), and the spatiotemporal $[Ca^{2+}]_i$ dynamics of δ-cells (7). In addition, ex vivo confocal imaging of the genetically encoded calcium indicator GcAMP3 in the mouse nodose ganglion identified serotonin as a central nervous system (CNS)-regulated signal released by islet vagal nerve that impacts insulin release (45).

These approaches complement the application of high-throughput single-cell sequencing technologies that show α-, β-, and δ-cells in molecularly and/or functionally distinct types (or states) (35). While further studies are needed to fully understand the physiological role of β-cell functional and transcriptional heterogeneity in health and disease, it is imperative to determine whether the observation of transcriptionally distinct β-cells represents unique and stable β-cell types/states or if these are in fact due to transcriptional fluctuations and/or noise caused by circadian rhythms (46), aging (47), or diabetes (48). Notably, recent evidence shows that β-cell heterogeneity can arise during pancreas development (49) and that different levels of the β-cell transcription factors PDX1 and MAFA have a significant impact on β-cell proliferation, function, and metabolism (50,51).

Electron Microscopy and the Ultrastructure of β-Cells

Occasionally, scientists need to visualize the anatomy of β-cell intracellular compartments at a much higher resolution than what is possible with light-based microscopes. To achieve this goal, scientists can use different applications

of electron microscopy (EM), including transmission EM (TEM) or scanning EM (SEM). One of the first studies that provided EM micrographs of cells in the islet was done in the 1950s by Paul E. Lacy, who described the unique anatomical features of major islet cell types and their hormone-containing granules (52); in subsequent studies investigators described the morphology of fine intracellular structures of β-cells, including cells from human embryos (53). Notably, the characteristic appearance of insulin granules (i.e., electron-dense core with a surrounding "halo" [Fig. 1]) is an artifact of specific chemical fixation processes, which can also affect cell and organelle morphology (54). We have applied large field of view SEM to map different regions of the human pancreas to reveal the ultrastructural details of islets and β-cells within their tissue context, including the double-basal membrane that envelops human islet capillaries (Fig. 1*A*) and the membrane invaginations that interconnect endocrine (Fig. 1*B*) and exocrine cells (Fig. 1*C* and *D*). In addition, we used TEM tomography to show the nanometer-scale anatomy of cytoplasmic extensions of δ-cells (7), the interface of a mouse β-cell with an islet capillary or peripheral islet nerves, and of β-cell intracellular structures such as a microtubule, endoplasmic reticulum (ER), and mitochondria (Fig. 1*E*–*G*). Furthermore, advances in the past 15 years have enabled the development of three-dimensional EM techniques, such as serial block-face EM, which allowed the reconstruction of small volumes of β-cells and neighboring cells (55). Similarly, focused ion beam SEM (FIB-SEM) was used to reconstruct the microtubular and organelle networks and insulin granules in isolated mouse β-cells (56), while FIB-SEM and cryo-EM were combined to reveal the neighborhood of insulin granules in a rat β-cell insulinoma cell line (57).

These examples highlight the need to apply multiple microscopy modalities to capture the different architectural, cellular, and functional aspects of β-cell biology. However, optimal islet cell EM is time-consuming, since it relies on thin pancreas sections (usually 50–250 nm thick) cut at random and that invariably generate a large collection of exocrine cells. This can be partially addressed by EM imaging of isolated islets; however, this approach eliminates their overall tissue context. To address this limitation and improve islet EM processing and imaging, we developed a "digital survey" step where epoxy-embedded pancreas blocks are scanned using X-ray microscopy (XRM) prior to tissue sectioning (Fig. 2*A*). This allowed us to determine the coordinates of target islets in the *x*, *y*, and *z*-axes within the tissue volume noninvasively and use these coordinates to guide sectioning of target islets for downstream TEM/SEM or imaging with other microscopy platforms (Fig. 2*A*) (58). While the application of these correlated microscopy platforms and analysis pipelines is extremely exciting, they pose significant logistical and technical challenges. For example, most of these imaging techniques require large investments in hardware, specialized technical know-how, or skills that may have limited availability and are only found in specific imaging core facilities and institutions. Furthermore, they can generate a significant amount of data that must be properly stored, processed, and annotated. Large microscopy data sets require integration with a dedicated analytical infrastructure at all levels (i.e., software, hardware, and skilled personnel). In fact, analysis of microscopy data increasingly demands that basic scientists learn programmatic language to interact with and analyze large-scale imaging data. Importantly, the tools created to generate and analyze data, associated data sets and metadata, analytical parameters, and visualization models need to be made publicly available for transparency and reproducibility efforts and to allow the free exploration of these multidimensional high-resolution maps of islet cell structure-function.

Breaking the Time Resolution Barrier—Quantifying Cell Age In Situ

Cells and large macromolecular structures in the CNS, such as the myelin sheaths, nuclear pore complex (NPCs), and nucleosomes, have remarkably low turnover rates (59). These findings were made using an in vivo ^{15}N-labeling strategy where ^{15}N-labeled pups were generated in utero and chased with a regular diet containing mostly ^{14}N for up to 12 months. Quantification of the ^{15}N-retaining peptides and protein lifetimes was determined by quantitative mass spectrometry analysis of bulk tissue (59). These experiments require large amounts of tissue mass (i.e., whole rat brains) and, although very informative, provide almost no clues regarding the spatial architecture and distribution of ^{15}N-labeled proteins besides the identity of the organ analyzed.

Analysis of stable isotope–labeled molecules and structures in situ can be achieved using multi-isotope mass spectrometry (MIMS) (also known as secondary ion mass spectrometry [SIMS]). In MIMS, the tissue surface is bombarded with a cesium ion (Cs^+) beam that ionizes and releases superficial secondary ions that are separated by their flight trajectories and quantified by mass detectors arranged in parallel positions (Fig. 2*B*). As a result, MIMS quantifies the elemental composition of stable isotope–labeled molecules and cells in situ. Here, the isotope data are represented by ratiometric mass images (e.g., ^{15}N-to-^{14}N) that provide the spatial distribution of labeled molecules (Fig. 2*B*). When combined with in vivo stable isotope–labeling approaches, MIMS has revealed the dynamics of protein and cell turnover in the gut epithelium, cardiomyocytes, and cochlear hair cells (60–62). More recently, we integrated XRM and SEM with MIMS (called MIMS-EM [Fig. 2*A* and *B*]) to determine the longevity of cells and protein supercomplexes in tissues of mice with high spatial resolution (58). The integration of these different microscopy modalities allowed us to bridge several scales of magnitude and focus the microscopes on cells or structures of different sizes in virtually any tissue

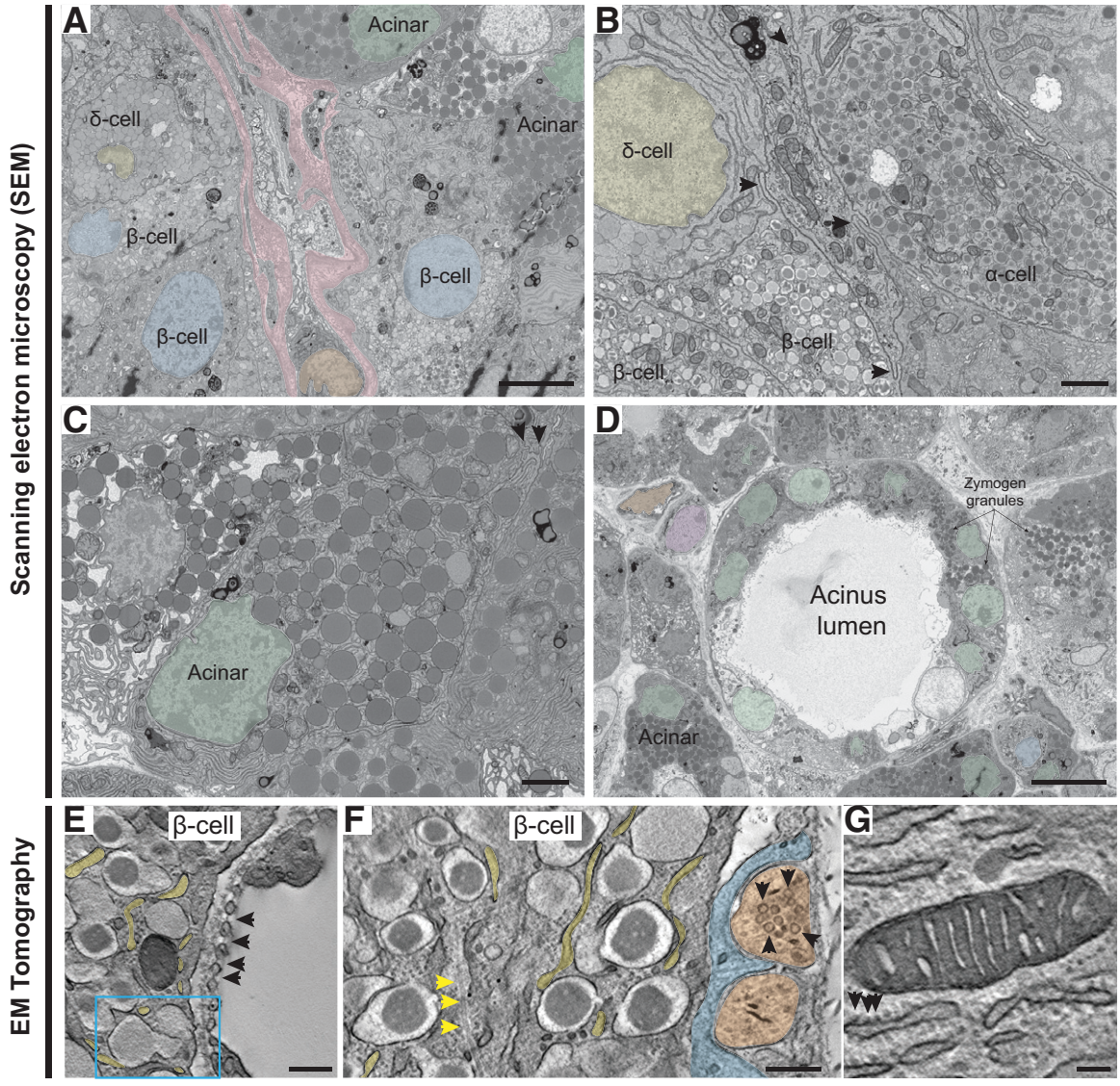

Figure 1—High-resolution EM imaging of cells in the pancreas. *A*: Cross section of the interface of endocrine cells, exocrine cells, and a capillary. β-Cells, a δ-cell, an endothelial cell (the vascular lumen has collapsed), and acinar cells are highlighted with blue, yellow, orange, and green nuclei, respectively. The thick double-layered basal membrane characteristic of the human islet vasculature is highlighted in faint pink color. *B*: Close-up of a human δ-cell (yellow nucleus) and partial sections of a β-cell (lower-left corner) and an α-cell (top-right quadrant) are shown and illustrate the unique appearance of somatostatin, insulin, and glucagon granules in EM micrographs, respectively. Black arrowheads indicate small plasma membrane invaginations that connect islet cells. *C*: Close-up of two human acinar cells with zymogen granules in full view. Cell highlighted by the green nuclei is intact, and small plasma membrane invaginations can be seen and are indicated by black arrowheads. Cell to the left has a compromised ultrastructure likely due to apoptosis and/or rupture during the sample-processing steps. *D*: Cross section of a human pancreatic acinus and lumen with surrounding acinar tissue (green nuclei), a collapsed capillary with an endothelial cell nucleus (orange nucleus), and stromal cells (purple nuclei) are shown. A single β-cell can be seen at the bottom-right corner (blue nucleus). *E*: TEM tomography reveals the ultrastructure of the interface of mouse β-cell with a fenestrated capillary. Cross sections of the endoplasmic reticulum (ER) are seen and highlighted in yellow. Insulin granules with their characteristic electron-dense insulin core and clear halo can be seen. Blue-bounded box highlights two insulin granules (one of them appears to be empty) in close proximity to and/or bound to the plasma membrane. Black arrowheads indicate the fenestrae in the capillary endothelium. *F*: TEM tomography of a β-cell cytoplasm in the periphery of an islet. Insulin granules are in full view. Yellow arrowheads indicate a single microtubule. Cross sections of peripheral islet innervation and wrapping Schwann cell filopodium cytoplasm are shown in orange and blue, respectively. Black arrowheads indicate neurotransmitter vesicles. Their spherical and non-disc-like shape suggest these are acetylcholine granules. *G*: TEM tomography of a single β-cell mitochondria with surrounding ER is shown. Black arrowheads indicate ribosomal complexes attached to the ER. Scale bars 5 μm (*A*), 1 μm (*B* and *C*), 10 μm (*D*), and 200 nm (*E* and *F*). Collection of human data was approved by the Institutional Review Board at the Salk Institute for Biological Studies, and animal experiments were approved by the Institutional Animal Care and Use Committee at University of California, San Diego (protocol no. S03172M).

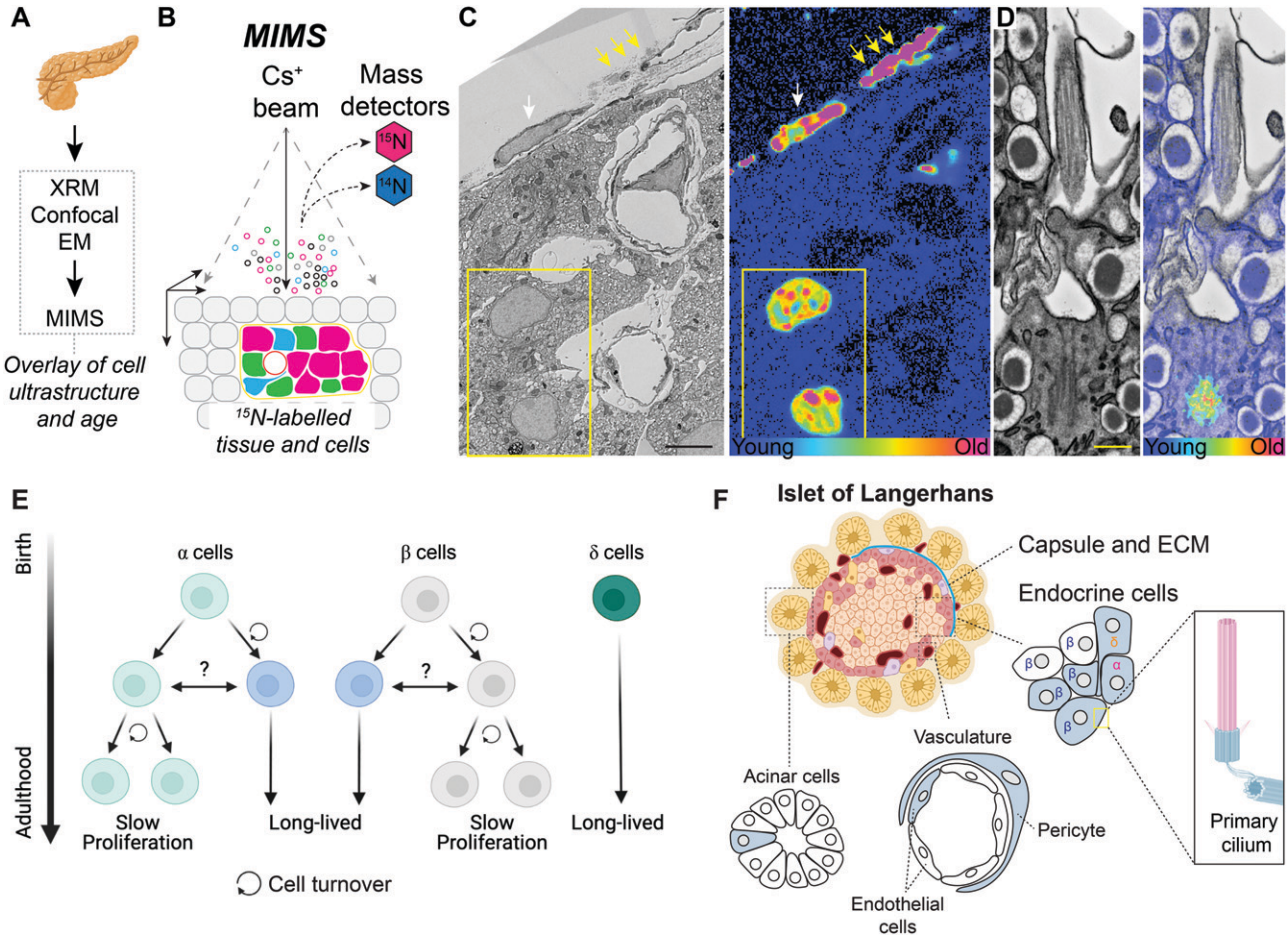

Figure 2—Imaging the age of cells in the pancreas with MIMS-EM. *A*: Application of XRM, confocal microscopy and/or EM correlated with MIMS imaging provides high-resolution information of cell structure overlaid with cell age quantification. *B*: In MIMS, a cesium ion beam (Cs^+) bombards the tissue surface to sputter secondary ions, which are then detected by parallel mass detectors. In mice labeled with the stable isotope ^{15}N and chased with ^{14}N, the ^{15}N-to-^{14}N ratio is used to quantify cell and/or structure age. *C*: Correlated EM (left) and MIMS (right) imaging of a section of an islet from a mouse labeled with ^{15}N until P45 and chased for 18 months. Area bound by the yellow box indicates the position of two long-lived β-cells. A long-lived fibroblast and collagen fibers associated with the islet capsule are indicated by white and yellow arrowheads, respectively. *D*: Correlated EM (left) and MIMS (right) imaging of a longitudinal cross section of a β-cell primary cilium from a mouse pulse labeled with ^{15}N until P21 and chased for 26 months. Note the localized retention of ^{15}N in the basal body. *E*: Heterogeneity in the turnover rates of postnatal α-cell and β-cell types results in cells with vastly different longevities, while most (and maybe all) δ-cells are remarkably long-lived. Long-lived α- and β-cells are shown in blue. Question marks indicate the possibility that long-lived α- or β-cells might replicate under unknown or stressful conditions or that proliferating cells might stop diving and become quiescent and/or postmitotic cells. *F*: Cartoon illustration summarizing the types of pancreatic structures that contain long-lived cell types (highlighted in blue). Relatively younger cells are shown in white. In the primary cilium, structures in the axoneme (highlighted in pink) turn over relatively faster than in the basal body. Scale bars: 2 μm (*C*), 200 nm (*D*). *E* and *F*: Made using BioRender.

while maintaining their biological context. Our main findings and their potential relevance to the β-cell biology and diabetes fields are discussed below.

Longevity of β-Cells

β-Cells are largely postmitotic cells that remain in this state throughout adulthood and therefore can be several decades old in humans (14,63). However, the observation that few β-cells could enter the cell cycle raised the possibility that some β-cells were relatively younger than others. To address this, we applied MIMS-EM to map the longevity of cells in mouse pancreas labeled with ^{15}N until 6 weeks of age and chased with ^{14}N for 18 months. MIMS of ^{15}N-labeled islet cells revealed a high degree of heterogeneity in the turnover of cells in the islet and in the exocrine compartment (Fig. 2*C*) (58). We found that most (∼60%) β-cells born by postnatal day 45 (P45) remained largely postmitotic for the rest of their lifetime, while the rest showed signs of proliferation (marked by the loss of nuclear ^{15}N during the chase period). Similar results were observed in α-cells, although the share of proliferative α-cells was smaller (58). As expected, MIMS-EM on ^{15}N-islet cells from a mouse labeled until weaning at P21 and chased for 26 months revealed a large fraction

(~75%) of young α- and β-cells, confirming previous reports that weaning is associated with increased rates of β-cell replication (64). Surprisingly, ~25% of postmitotic α- and β-cells were established prior to weaning (58), whereas no evidence of significant turnover in the δ-cell population was found (58).

These results suggest that old and young β-cells could represent different types of β-cells, where younger cells may characterize a replication-prone population. However, lineage-tracing experiments show that β-cell mass homeostasis does not result from the expansion of a single β-cell clone (65). Therefore, a likely explanation is that the younger β-cells observed in our experiments represent multiple β-cell clones/lineages that may have a higher likelihood of replication during adulthood. Future experiments are needed to determine the exact molecular identity of old and young β-cells and their lineage, including whether younger β-cells have a similar immature transcriptional signature characteristic of replicating β-cells (66), and whether long-lived β-cells can enter the cell cycle under stress (Fig. 2*E*). Importantly, differences in the longevity of β-cells may explain the heterogeneous expression of aging markers in β-cells and their connection to the onset of age-dependent senescence and diabetes (67,68).

Long-lived Macromolecular Complexes in the Pancreas

Our MIMS-EM studies recently revealed that longevity of proteins and cells is not exclusive to the CNS, where neurons contain long-lived proteins (LLPs) that can last a lifetime (59). Accordingly, we used MIMS-EM to pinpoint the location of long-lived structures in the CNS, including the peri-capillary extracellular matrix (ECM), myelin sheaths and the primary (58). Surprisingly, we found that this remarkable structural longevity was also found in somatic organs, including the pancreas. Here, we have shown that most of the intra-islet ECM is rapidly replaced while the islet capsule ECM is remarkably long-lived (58) (Fig. 2*C*). The islet capsule contains long-lived elements whose changes in stability are observed in T1D (69), and the accumulation of long-lived ECM components may contribute to the vascular fibrosis phenotype seen in aging and T2D (6). We also found that, like in neurons, the basal body of the primary cilium in β-cells contains a remarkably long-lived structure located at or below the primary cilium transition zone (58) (Fig. 2*D*). While the identity of the long-lived molecules in this specific region remains a mystery, its structural resilience may signal that long-lived macromolecules in the basal body may be important to support the structural stability of this organelle and maintain β-cell polarization and normal cell function for long periods of time.

These findings support previous results that β-cells and other pancreatic cell types are long-lived cells (14,63). Moreover, these new spatially resolved maps of cellular longevity show that long-lived cells are distributed throughout the pancreas and found in other major organ structures (e.g., liver), each one formed by a mosaic of cells and macromolecular structures of different ages (Fig. 2*F*). Although the physiological relevance of long-lived macromolecular components and cells remains to be determined (discussed in detail below), it is striking that most islet cells in the pancreas become postmitotic cells early in postnatal life and survive for many decades with little to no turnover. Future research will focus on understanding the molecular mechanisms that grant this remarkable longevity to macromolecules, organelles, and cells that remain in place and mostly functional for many decades despite years of exposure to metabolic, environmental, and/or genetic insults.

Does β-Cell Age Really Matter? Age Mosaicism as a Basic Multiscale Organization of Islet Architecture and Function

In 1959, while studying the organization and behavioral pattern of termites in their nests, Pierre Grasse observed the coordinated behavior of the termite swarm despite the chaotic behavior of individual insects. These observations led to the term "Stimergie" (or stigmergy), which describes the indirect coordination of a swarm's behavior in response to environmental modifications left by individual members of the colony (70). In this context, modifications refer to the deposition of signals (i.e., pheromones) or physical modifications of the environment (e.g., carving of tracks) as each member executes a specific task. These signals remain in the environment for long periods of time and are detected by other members long after the first member has passed and ultimately coordinate swarm behavior and function (70).

A similar concept could be applied to explain tissue development and homeostasis. In fact, Alan Turing (after breaking the Enigma code during the Second World War) developed mathematical models that predict how identical cells can interact with gradients of chemical morphogens present in space to achieve determined cell fates over time. Turing proposed that the "residency time" of molecules is an important variable in determining tissue morphogenesis (71). Turing's theory has been validated almost entirely (72) and together with Grasse's work, his calculations provide the theoretical scaffold to support the idea that biological systems are organized by a basic principle where longevity of specific macromolecular components contributes to or dictates biological behavior. This principle would be found in single protein complexes, organelles, cells, organs, and whole organisms and in their spatial interactions. This organization pattern entails the multidimensional arrangement and interaction dynamics of relatively younger components with long-lived structures, in a pattern we termed "age mosaicism" (58).

Like the pheromones deposited by termites or Turing's hypothetical morphogens, LLPs and long-lived cells would act as the "stigma" in cells, cells clusters, and in tissues. This protein and cellular longevity would create

macromolecular beacons encoding a "biological GPS" that propagates protein complex, cell, or tissue structure-function to newer generations of components formed during adulthood and that were not exposed to important developmental cues that shape structure and function. I would like to propose the model of age mosaicism as an integral part of pancreas organogenesis, β-cell structure-function, and glucose homeostasis. This idea is supported by our understanding of how different types of cells in the pancreas integrate physical and chemical signaling cues during development to successfully achieve and maintain a differentiated mature-cell state that supports adult organ function and glucose homeostasis (reviewed in refs. 73,74).

At the cell level, age mosaicism would play a role in both the developing and adult pancreas. Similar to termites (70), the migration of delaminating lineage-committed cells from endocrine progenitor cell niches and their correct establishment within the developing pancreas architecture would be guided by the chemotaxic action of long-lasting molecules deposited on the pancreas ECM network by other developing cells (Fig. 3*A*) (75). In turn, the ECM would be deposited by "trailblazing" fibroblasts, cells that would be born very early in life to shape the organ mesenchyme by creating an ECM scaffold (i.e., tracks and tunnels) to allow cell migration and the correct positioning of differentiating endocrine cells. Accordingly, remarkably long-lived fibroblasts are found in close proximity to islets (58); in another example, extremely long-lived β-cells—the first cells to acquire a mature state in the developing pancreas—could act as signaling hubs that facilitate cell migration or differentiation by promoting lineage-specific programs (Fig. 3*A*). These cells would have a role similar to that of long-lived and mitotic $Ngn3^{+}$ cells in the developing pancreas (76).

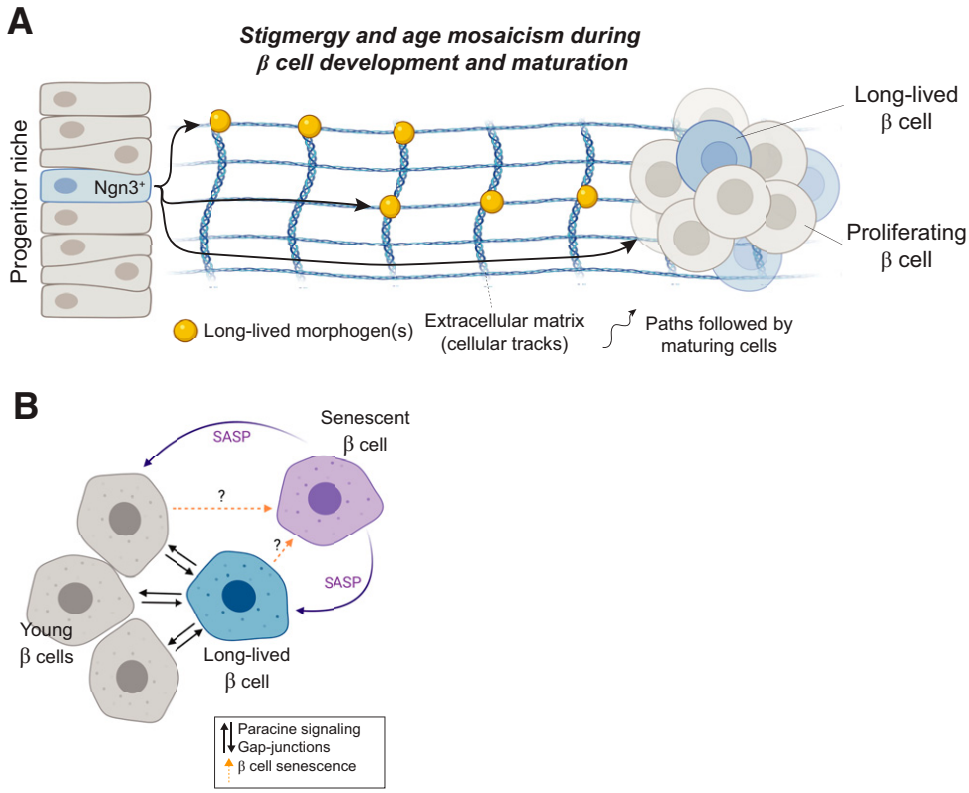

Figure 3—Age mosaicism and potential implications for islet cell development, function, and homeostasis. *A*: Theory of stigmergy and age mosaicism applied to explain β-cell development and maturation process. Neurogenin 3 $(Ngn3)^{+}$ endocrine progenitors (which can be long-lived) give rise to endocrine fate–committed cells that delaminate and follow physical tracks outlined by the ECM in the developing pancreas mesenchyme and/or the chemical trace from morphogens with different longevities anchored to the ECM. ECM would be deposited by fibroblasts, which would "pave the way" for tissue morphogenesis and prepare, together with vessels (not shown), the mesenchyme for endocrine fate–committed cells to establish primordial islets. Some fibroblasts would remain in the tissue throughout adulthood in a quiescent and/or postmitotic state. As the initial islet architecture is stablished over time, a growing number of β-cells would exit the cell cycle, mature, and become postmitotic cells. This process is expected to start in utero and continue until early adulthood, when most β-cells are postmitotic. *B*: A long-lived β-cell (blue) as signaling hub in the islet. Mature-state long-lived β-cells would relay information to neighboring and younger β-cells (light gray) via paracrine signaling and/or gap junctions. This would ensure that β-cells born during adulthood acquire the necessary functional thresholds to meet the metabolic demands of adult glucose homeostasis. In purple a senescent β-cell is shown, and purple arrows indicate the contribution of senescence-associated secretory phenotype (SASP) factors that impact β-cell function and/or survival. Question marks indicate unanswered questions relating to which β-cells (old vs. young) are more likely to become senescent β-cells. Made using BioRender.

In the adult pancreas, long-lived β-cells would serve as structural "guides" for placement of β-cells generated by replication of neighboring β-cells and/or they would communicate (via paracrine and gap junction signaling) with younger β-cells born during adulthood to dictate the maturation and fine-tuning of function of younger β-cells (Fig. 3*A* and *B*). This is analogous to what has been proposed for hub and/or leader β-cells, which are molecularly distinct β-cells that can affect neighboring β-cell function (31,42). This could explain how replicating (and initially immature) β-cells that are generated in the adult islet environment—long after the early-life signaling events that drive β-cell maturation take place (64)—could achieve a mature and functional state that meets the physiological and metabolic requirements of an adult organism. With advanced age, it is possible that long-lived β-cells may accumulate DNA damage after being exposed for several years to reactive oxygen species, or perhaps younger β-cells accumulate damage as a result of repeated cell divisions that would ultimately contribute to β-cell senescence (Fig. 3*B*) (67,68). The exact molecular signature of long-lived β-cells, including whether they share similarities with hub/leaders and/or virgin β-cells or are more or less likely to become senescent, remains to be determined.

At the organelle and protein level, age mosaicism could play a role in the maintenance of islet cell polarization and signaling whereby LLPs present in the basal body would stabilize the primary cilium structure to maintain long-term integrity of cilium-associated microtubules and signaling pathways and prevent entry into the cell cycle. A similar role for LLPs has been suggested in neuronal NPCs, where LLPs provide structural stabilization and integrity. Accordingly, age-associated loss of LLPs underlies the decline in nuclear function and the collapse of nuclear-cytoplasmic barrier control (77). Notably, age mosaicism is observed within the pool of secretory insulin granules, where relatively younger insulin granules are more motile than older granules and more likely to be released under stimulated conditions (78).

Finally, this age mosaicism organization theory is expected be relevant to organization and function of all tissues. Age mosaicism has recently been observed in the vastly different lifetimes of biological elements and systems, from the varying half-lives of NAD^+ across tissues (79) to the long-term association of transcription factors with the chromatin to determine cell phenotype (80), to the longevity of NPCs, histones, and mitochondria (58,59,81), and in tissue-specific T-cell types (82). In each of these examples, the longevity of specific molecules and cells influences the function of important homeostatic systems across the body in a tissue- and cell type–specific manner.

Conclusion

The increasing availability and sensitivity of single-cell phenotyping technologies allows us to quantify various aspects of β-cell biology in health and disease. These techniques have begun to reveal the complex and multidimensional nature of cell organization patterns, molecular phenotypes, and signaling pathways that support β-cell development and function and glucose homeostasis. These observations include the fact that the vast majority of β-cells, like many other cells in the islet, are long-lived cells. This remarkable cellular longevity requires a fundamental resiliency, maintaining cellular and energy homeostasis, and retaining functional cellular organization for significantly longer than previously suspected. Therefore, it is crucial for us to understand the mechanisms that have evolved to support β-cell longevity and how long-term β-cell homeostasis mechanisms are impacted by diabetes. These efforts would allow the development of approaches to enhance β-cell resiliency mechanisms to preserve existing β-cells in patients with T1D and T2D, while revealing new ways to improve the resilience and maturation of stem cell–derived β-cells to achieve an effective and long-lasting treatment of T1D.

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