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Transcriptional Noise and Somatic Mutations in the Aging Pancreas

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The underlying mechanisms and functional significance of pancreatic β cell heterogeneity are an intensive area of investigation. In a recent *Cell* paper, Enge and colleagues (2017) performed single-cell RNA sequencing of human pancreatic cells and concluded that with age, pancreatic cells become transcriptionally noisy and accumulate somatic mutations.

An emerging theme in the study of pancreatic β cells, the cell type whose failure is central to diabetes, is their heterogeneity (Avrahami et al., 2017; Roscioni et al., 2016). For example, a minority of β cells were shown to act as crucial hubs that synchronize the activity of other β cells in the islets of Langerhans, at least in mice (Johnston et al., 2016), and four antigenically distinct β cell subtypes were recently identified in humans (Dorrell et al., 2016). However, the mechanisms underlying β cell heterogeneity remain unclear: we do not know if heterogeneity reflects stable cellular subtypes or dynamic cell states; whether it is the result of microenvironmental factors such as location within the islet or even alternative fetal origins; whether β cell heterogeneity has epigenetic underpinnings; and most importantly, to what extent β cell heterogeneity plays a role in diabetes. Novel single-cell omics technologies have begun to provide answers to these questions. Indeed, there has been a recent avalanche of papers describing single-cell RNA-sequencing profiles of β cells from mice and humans, as well as the beginning of single-cell proteomics (Wang et al., 2016).

In a recent *Cell* paper, Enge and colleagues analyzed the transcriptomes of 2,544 individual pancreatic cells from eight human organ donors, ranging in age from 1 month to 54 years, and reported several provocative insights into the nature of pancreatic cell heterogeneity (Enge et al., 2017). One key finding is that transcriptional noise in the pancreas—defined as the variation of expression levels of individual genes among cells of the same individual—is increased with age. This observation is consistent with a view of aging as a process involving stochastic, rather than programmed, changes in gene activity. Related to this, the authors noticed that individual endocrine cells in older people were more likely to express irrelevant hormone genes, such as glucagon in β cells or insulin in α cells. The presence of such bi-hormonal islet cells is a well-known phenomenon, associated with β cell dysfunction. In fact, one model of β cell failure in type 2 diabetes suggests that it stems from loss of β cell identity due to metabolic stress, including gained expression of irrelevant hormone genes (Talchai et al., 2012). The findings of Enge and colleagues on bi-hormonal cells suggest an intriguing link between age-dependent transcriptional noise and compromised islet cell identity. Interestingly, bi-hormonal cells are also abundant in fetuses and in suboptimal differentiation protocols of embryonic stem cells. Thus, it would be important to establish whether fetal, immature β cells are also transcriptionally noisy.

A second major finding by Enge and colleagues is the identification of somatic mutations in individual pancreatic cells, including β cells. Somatic mutations have been typically associated with cancer, although genetic mosaicism in the brain recently emerged as an important phenomenon (McConnell et al., 2017). The presence of mutations was inferred from analysis of RNA sequencing data (rather than sequencing of genomic DNA), which limits the analysis to exonic sequences. Pancreatic cells had up to a few thousand exonic somatic mutations per cell, a rate five times higher than in the brain. The mutational load in the pancreas, like transcriptional noise, was positively correlated with age. The most abundant mutations in the pancreas were substitutions of cytosine typical of errors known to occur during the repair of oxidative stress-induced DNA adducts. Consistent with oxidative damage as a driver of somatic mutations in the pancreas, the authors found high levels of oxidized guanine (8-oxo-deoxyguanosine) in pancreatic islets. Oxidized guanine is mutagenic because it can mispair with adenine. Notably, it is not clear if mutant transcripts were generated from damaged (oxidized) DNA or from a mutation introduced during damage repair. Overall, the results suggest that oxidative stress leads to age-related accumulation of somatic mutations in post-mitotic pancreatic cells (Figure 1).

The work by Enge and colleagues raises many fascinating questions regarding the scope, the causes, and the consequences of transcriptional noise and somatic mutations. Since somatic mutations were inferred from RNA sequencing, the study was of course blind to mutations that occur in non-transcribed regions, especially in regulatory elements that affect transcription. Uncovering such mutations will require true genomic sequencing at the

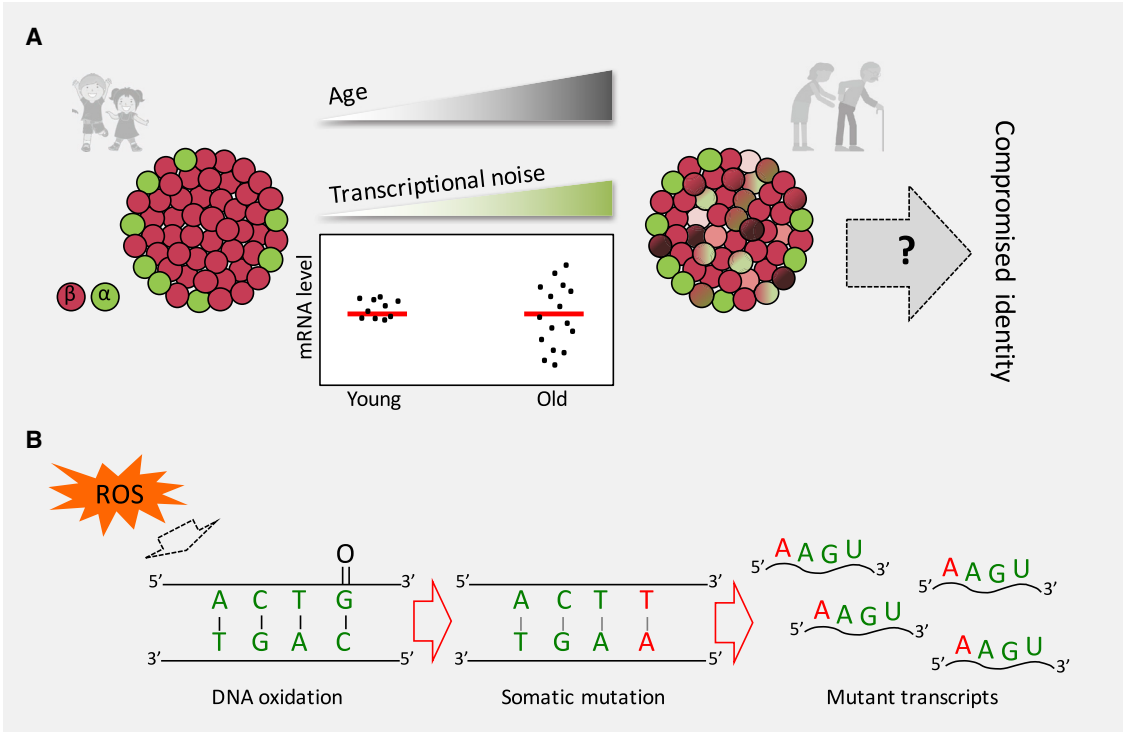

Figure 1. Schematic of the Findings of Enge et al., Based on Single-Cell RNA-Sequencing of Pancreatic Cells
(A) Age-related transcriptional noise and expression of illegitimate genes in islet cells.
(B) Accumulation of somatic mutations in individual post-mitotic pancreatic cells as a consequence of oxidative damage to DNA. Mutant transcripts could be generated either from oxidized DNA or upon repair and fixation of mutation.

single-cell level, given the lack of clonal expansion in the adult pancreas. In addition, other types of mutations remain to be identified in the pancreas. This is particularly interesting given the occurrence of double-strand DNA breaks in β cells in type 2 diabetes (Tornovsky-Babeay et al., 2014), because such breaks are typically repaired by error-prone non-homologous end-joining leading to insertions and deletions. It will be interesting to find if aging and diabetes are associated with different types of mutations.

What factors are driving transcriptional noise and somatic mutations in the pancreas of aged individuals? While mutations are by nature irreversible and therefore are expected to accumulate with age, the transcriptome is being replaced continuously, and therefore a mechanism must exist for enhancing noise in older age. Enge and colleagues did not find support for the attractive idea that somatic mutations (at least in transcribed genes) drive transcriptional noise; alternative explanations may relate to epigenetic changes, such as age-related alterations in DNA methylation or the pattern of histone modifications (Avrahami et al., 2015).

Finally, the study leaves open the functional significance of the observed transcriptional noise and somatic mutations, in particular with regard to β cell failure in diabetes. It is intuitive to assume that transcriptional noise would cause deterioration of function; however, mechanisms exist for dampening the impact of noise (for example, nuclear retention of RNA, serving to buffer fluctuations in transcript levels) (Bahar Halpern et al., 2015), and there are also suggestions that heterogeneity due to transcriptional noise is an ancient mechanism ensuring survival of a population of cells under stress. Therefore, addressing the impact of transcriptional noise on β cell function is an important future challenge. Somatic mutations could lead to permanent functional decline of β cells in type 2 diabetes and potentially also to the generation of neo-epitopes driving autoimmunity in type 1 diabetes, but again this will require a direct experimental proof. The analysis of transcriptional noise and somatic mutations in β cells from patients with diabetes is an obvious next step in the long path to understanding the nature of β cell failure. The study of Enge and colleagues provides a solid stepping stone to embark on this journey.

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When Cancer Cells Are Given Lemo[NH_3]s, They Make Lemo[NH_3]ade

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In normal physiology, end-products of metabolism are excreted from the body. In tumors, these metabolic wastes accumulate due to deregulated metabolism and vascular insufficiency. Spinelli et al. (2017) show that breast cancer cells adapt to ammonia buildup by recycling it for amino acid synthesis, which can support cancer cell growth.

In tumors, disruptions in tissue architecture and inefficient vascularization often lead to nutrient exhaustion and accumulation of metabolic wastes. To tackle these challenges, the cells in a tumor rewire their metabolic processes to facilitate malignant cell survival and tumor growth. It is becoming increasingly appreciated that this includes the capture of waste products and their recycling as biosynthetic building blocks. For example, lactate, classically considered as the unneeded output of glycolytic metabolism, accumulates in tumor tissue due to the high rate of glycolysis in the malignant cells (i.e., the "Warburg effect"). Recent studies now indicate that lactate contributes to tumor progression in various ways, including as a carbon source to feed mitochondrial metabolism (Faubert et al., 2017; Hui et al., 2017). Similarly, ammonia (NH_3) can accumulate at millimolar concentrations in the tumor microenvironment. Whether ammonia has other fates and how it is metabolized in and cleared from a tumor remain open questions. Yang et al. recently showed that ammonia is captured by stromal support cells and released to ovarian cancer cells as glutamine (Yang et al., 2016). Even more directly, Spinelli et al. now reveal a new mechanism for cell-autonomous ammonia assimilation and utilization in breast cancer cells (Spinelli et al., 2017).

Mammalian cells are restricted to a limited number of strategies for detoxifying ammonia. The primary mechanism is through the urea cycle, in which ammonium and bicarbonate are captured by carbamoyl phosphate synthetase I (CPSI) to form carbamoyl phosphate. This pathway is largely restricted to the liver. Secondary to the urea cycle, glutamine synthesis has been considered a backup mechanism for ammonia detoxification. In this process, ammonium is added to glutamate to produce glutamine, in an ATP-driven reaction catalyzed by glutamine synthetase (GS). In addition, there exists a third enzyme capable of ammonia assimilation: glutamate dehydrogenase (GDH). Previous studies on GDH have focused on its role in the oxidative deamination of glutamate. In this context, GDH is important for anaplerosis through alpha-ketoglutarate (αKG) generation (Csibi et al., 2013) and in redox regulation due to its coupling to NAD(P)H production (Jin et al., 2015). However, the directionality of the GDH reaction is dependent on the metabolic state of the cell and the availability of nutrients in the tumor microenvironment, following Le Chatelier's principle. In the study by Spinelli et al., the authors illustrate this phenomenon and unravel surprising outcomes of this fundamental principle.

Using estrogen receptor-positive (ER+) breast cancer cells, Spinelli et al. first determined the fate of the amide group of glutamine by ^{15}N-isotope tracing and mass spectrometry. Expectedly, asparagine and nucleotides, building blocks for biosynthesis that are linked to direct glutamine deamidation, acquired the ^{15}N isotope from glutamine. But to their surprise, proline, aspartate, branched-chain amino acids (BCAAs), and glutamate, metabolites that had not been previously linked to the biosynthetic role of the amide-nitrogen of glutamine, also incorporated the ^{15}N isotope. Glutamine deamidation can also occur through glutaminase (GLS)-mediated ammonia liberation during glutaminolysis (Figure 1). Using a glutaminase inhibitor, the authors found a significant decrease in the ^{15}N