will lead to higher methane production and to convection-enhanced diffusion becoming an increasingly significant mechanism for releasing this methane to the atmosphere, constituting a newly recognized positive feedback to climate warming. In the meantime, Poindexter and colleagues' results underscore the need to consider wetlands and lakes as dynamic systems, even on clear, calm nights. ■

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PHYSIOLOGY

Pancreatic β-cell heterogeneity revisited

Two analyses of insulin-producing β-cells reveal differences in what has long been considered a homogeneous population. These differences might reflect changes during maturation or ageing, or distinct cell lineages. SEE LETTER P.430

SUSAN BONNER-WEIR & CRISTINA AGUAYO-MAZZUCATO

Diabetes is associated with an inadequate supply of functional pancreatic β-cells, which normally maintain healthy blood-glucose levels by secreting insulin in response to raised levels and shutting off secretion when glucose is scarce. These cells have been regarded as a single, homogeneous population, even though studies have shown that β-cells are functionally immature in the neonatal period immediately after birth[1,2], and that adult β-cells are not all identical[3,4]. Now, two papers (Bader *et al.*[5] on page 430 and Dorrell *et al.*[6] in *Nature Communications*), identify genetic markers that allow subpopulations of β-cells to be isolated and studied. These studies are bound to revive interest in β-cell heterogeneity and highlight the relevance of this research area for understanding and enhancing cell-replacement therapies for diabetes.

Although all adult β-cells may seem the same when analysed using stains for insulin, subpopulations can differ in terms of insulin secretion[3,4] and insulin-expression levels[7], length of chromosome end structures called telomeres[8] (which correlates with the number of times the cell has divided) and expression of the gene *p16Ink4a*, a marker and effector of β-cell growth arrest (senescence)[9]. Bolstering the idea that this heterogeneity is functional are differences in β-cell sensitivity to glucose[10] and the fact that the cells can be recruited by increased glucose levels to adopt biosynthetic[11,12] or active secretory states[12–15], adapting to the demand for more insulin secretion.

Pancreatic islets are micro-organs composed of several hormone-secreting cell types, including β-cells. During the development of islets, cells are organized into a 3D architecture through a mechanism called planar polarity. Bader *et al.* investigated β-cell expression of a planar-polarity protein called Flattop (Fltp) in mice.

The authors replaced the gene encoding Fltp with a gene that encodes a fluorescent reporter protein dubbed FVR, so that cells that normally express Fltp fluoresced. In this way, they defined two β-cell subpopulations — proliferative immature cells that did not express Fltp or fluoresce (FVR$^-$), and

50 Years Ago

It is not surprising that the Prime Ministers of France and Britain should have been grateful for a few crumbs of agreement to fill out the communique after a recent meeting in London. By all accounts, British membership of the European Economic Community was not a fruitful topic of conversation. It is therefore paradoxical that the two Prime Ministers should have considered this a suitable time for a decision in principle that the tunnel beneath the English Channel should in due course be built … The case for a tunnel is wearing thin. Alternative ways of crossing what is, after all, a narrow strip of water have become more attractive … It looks very much as if the two governments may be making the right decision at the wrong time … it is to be hoped that the two governments will have another close look at the wisdom of the course to which they have committed themselves before the digging of a tunnel actually begins.
From *Nature* 23 July 1966

100 Years Ago

Like ourselves, the industrial cities of the United States are beginning to realise the serious economic and hygienic effects caused by the unscientific combustion of coal … Dr W. F. M. Goss has contributed to a paper on "Smoke as a Source of Atmospheric Pollution" … The author is not very optimistic in his outlook, for he considers that a revolution in practice which will result in the elimination of existing sources of atmospheric pollution is not to be expected "because present-day knowledge is insufficient to supply necessary means" … We are throwing away … a valuable inheritance which should belong to coming generations, and which they will never be able to recover.
From *Nature* 20 July 1916

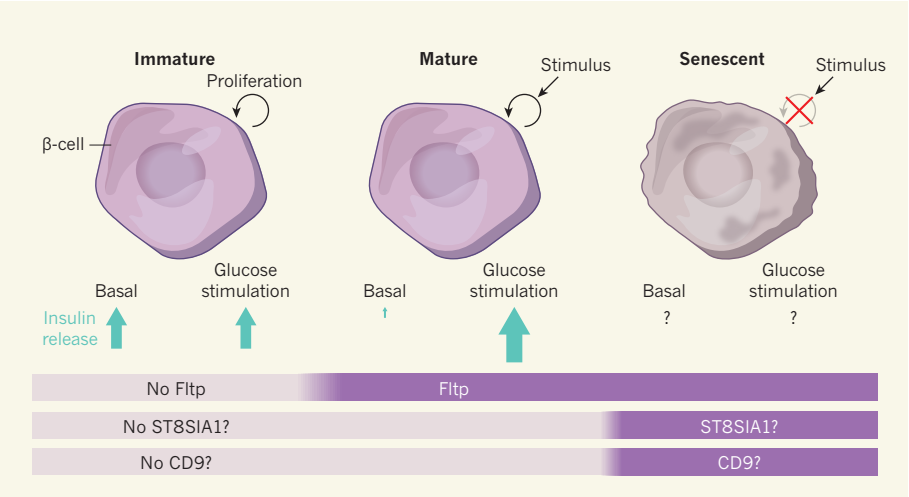

Figure 1 | Separating β-cell subtypes. The insulin-secreting β-cells of the pancreas are thought to renew slowly, so, at any one time, cells of different ages will be present in the tissue. Immature cells are highly proliferative and do not respond robustly to glucose stimulation, but secrete some insulin even at basal (low) blood-glucose levels. Mature cells proliferate in response to external stimuli and release insulin robustly, secreting little or no insulin under basal conditions. Old cells cease to proliferate (they become senescent) and may be dysfunctional. Bader *et al.*[5] show that immature cells do not express the protein Flattop (Fltp), but mature cells do. Dorrell *et al.*[6] find that subsets of β-cells express the proteins ST8SIA1 and/or CD9. Although it was not tested, it could be hypothesized that these cells might be senescent, because they show impaired glucose-stimulated insulin secretion compared with the β-cells from the same samples that lack these markers.

mature, quiescent fluorescing cells (FVR^+). Eighty per cent of β-cells were FVR^+ in adult mice, as were about 50% of other islet cells. It is of considerable interest that 20% of adult cells are FVR^-, because it has been reported[3,10] that 20% of β-cells in adult rodents are unresponsive to glucose.

The authors next separated and further analysed each subpopulation for gene expression, ultrastructure and insulin secretion. These experiments supported the idea that Fltp is a marker of mature β-cells. Furthermore, an *in vitro* tracing analysis confirmed that β-cells progress from FVR^- to FVR^+ over time. FVR^- cells proliferated more than FVR^+ cells during the normal β-cell population expansion seen during the neonatal period and pregnancy. By contrast, when mice were fed a high-fat diet, which increases the demand on β-cells, cells in the FVR^+ subpopulation increased in size, thus presumably increasing their insulin-secreting capacity.

Fltp seems to be a marker, rather than an effector, of the β-cell transition from immature and proliferative to mature and quiescent — deletion of Fltp in mice did not impair β-cell development, proliferation or maturation. Adult mice that lacked the protein had normal glucose tolerance, insulin sensitivity and levels of pancreatic insulin and glucagon, a hormone that raises blood glucose. However, Bader *et al.* found that activation of a signalling cascade called the Wnt/planar cell-polarity pathway, of which Fltp is a downstream effector, enhanced human and mouse β-cell maturation *in vitro*. Moreover, manipulating cells into 3D aggregates had the same effect, indicating that planar polarity is important for β-cell maturation. In the future, the development of specific antibodies that bind Fltp will facilitate a range of experiments to study its role in the human pancreas.

In the second study, Dorrell *et al.* provide further evidence for the existence of β-cell heterogeneity, this time in humans. Previously[16], the same group used antibodies that bind cell-surface proteins to identify hormone-secreting cell types in partially purified isolated human islets. In the current paper, they report the development of two more antibodies that allowed them to distinguish four subtypes of β-cell in the adult human pancreas. The antibodies bind molecules whose role in β-cells is unknown — CD9, a cell-surface glycoprotein that in other systems suppresses cell proliferation, and ST8SIA1, a membrane-bound glycosphingolipid involved in cell adhesion and cell growth.

The authors found consistent proportions of the four β-cell subtypes in healthy adults. Expression of genes crucial for β-cell identity was similar in each subgroup, but expression of around 100 other genes of mostly unknown function in β-cells differed. Cells that expressed both CD9 and ST8SIA1 released more insulin in basal conditions and were less responsive to glucose stimulation than those negative for both markers. This impaired responsiveness may be relevant for disease, because β-cell function is impaired in type 2 diabetes, and Dorrell and colleagues showed that the two ST8SIA1-expressing subtypes accounted for 45% of β-cells in people with this disease, compared with 18% in healthy adults.

What is the biological reason for β-cell heterogeneity? We have hypothesized[17] that young β-cells differ from those in the early stages of maturation or in midlife, those that are mature and have stopped dividing, and those that are old or dying. If this is true, the observed subtypes might reflect different stages of the β-cell life cycle (Fig. 1).

Although Bader *et al.* showed that mouse cells transition from the Fltp-lacking to the Fltp-expressing subgroup over time, it is unclear whether the four human β-cell subtypes observed by Dorrell and colleagues have a temporal relationship or are independent lineages. It also remains unclear whether the increased proportion of ST8SIA1-expressing subtypes in people with type 2 diabetes is the result of selective loss of functionally active β-cells that lack ST8SIA1, or arises because of progressive dysfunction of β-cells owing to the cellular stress of diabetes. Regardless of whether the four human subtypes are separate lineages or sequential stages, their heterogeneity should be considered in research that aims to generate optimal cells for β-cell replacement therapy, because these subgroups may differ in their proliferative capacity or vulnerability to cellular stress.

Together, these papers revisit and underscore the need to consider β-cells as a heterogeneous population comprising cells at different stages of development with different functional and proliferative characteristics. Thanks to the current studies, the tools to explore these differences further are now available. ■

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