Review

β-Cell regeneration: the pancreatic intrinsic faculty

Renaud Desgraz[a,b], Claire Bonal[a,c] and Pedro L. Herrera

Department of Cell Physiology and Metabolism, University of Geneva Faculty of Medicine, 1 rue Michel-Servet, 1211 Geneva 4, Switzerland

Type I diabetes (T1D) patients rely on cumbersome chronic injections of insulin, making the development of alternate durable treatments a priority. The ability of the pancreas to generate new β-cells has been described in experimental diabetes models and, importantly, in infants with T1D. Here we discuss recent advances in identifying the origin of new β-cells after pancreatic injury, with and without inflammation, revealing a surprising degree of cell plasticity in the mature pancreas. In particular, the inducible selective near-total destruction of β-cells in healthy adult mice uncovers the intrinsic capacity of differentiated pancreatic cells to spontaneously reprogram to produce insulin. This opens new therapeutic possibilities because it implies that β-cells can differentiate endogenously, in depleted adults, from heterologous origins.

The different cell types of the adult pancreas, their function and their origin

The mammalian pancreas is a mixed gland of the digestive tract, composed of about 99% of exocrine cells and 1% of endocrine cells. Acinar and ductal cells constitute the exocrine part. Acinar cells secrete digestive enzymes that are forwarded to the duodenum along the ductal excretory tree. Food ingestion triggers nerve and hormonal enteric responses with the release of gastrin (by gastric G-cells), secretin (by duodenal K-cells) and cholecystokinin (by duodenal L-cells), resulting in exocytosis of zymogen granules by pancreatic acinar cells [1]. The endocrine compartment is formed by the islets of Langerhans that are dispersed within the exocrine parenchyma. These are aggregates of four different peptide-hormone-producing cell types, densely vascularized and innervated. In rodents the core of the islets is formed by insulin-producing β-cells (80% of the islet cells) surrounded by the three other cell types, namely α-, δ- and PP-cells, which secrete glucagon, somatostatin and pancreatic polypeptide (PP), respectively. A rare fifth cell type, ε-cells, found mainly during pancreatic development, produce ghrelin [2]. Together they not only control blood glucose homeostasis but also contribute to the regulation of food intake and gastric emptying. Briefly, blood glucose levels rise following food intake, stimulating β-cells to secrete insulin. Circulating insulin in turn represses glycogenolysis and neoglucogenesis and promotes glucose uptake and processing by muscle cells and adipocytes. Conversely, when blood glucose is low, glucagon secreted by α-cells stimulates hepatic glycogenolysis and neoglucogenesis, thereby restoring normoglycemia. Somatostatin and PP negatively regulate β-, α- and acinar cell secretion; ghrelin represses β-cell secretion [3,4].

The definitive adult pancreatic cell mass seems to be determined during development by a small pool of endodermal Pdx1+ progenitor cells distributed in two primordia (dorsal and ventral) [5]. These progenitor cells proliferate and differentiate into two distinct major lineages: endocrine precursors expressing neurogenin3 (*Neurog3*), and Ptf1a+/Mist1+ exocrine progenitors, each with restricted differentiation potential [6,7]. Considered individually, each *Neurog3*-expressing cell is unipotent and is specified to one of the four islet endocrine cell types [6]. During development it appears that temporal competence windows determine or influence the fate of *Neurog3*+ cells: the earliest *Neurog3*-expressing cells tend to mainly differentiate into α-cells [8]. The early endocrine cells aggregate into proto-islet-forming pools, whose size predetermines the final size of the islets [6]. These early developmental steps are crucial because any alteration of the β-cell mass can result in faulty blood glucose control, leading later in life to glucose intolerance and diabetes (for a review on the concept of 'fetal origin of adult diseases' see [9,10]). While this review was in press, a new paper reported the first evidence of non-genetic transmission of high-fat diet metabolic sequelae from father to offspring [11].

Towards cell replacement therapy

In normal healthy conditions β-cells have a long life-span with a low proliferation rate [6,12]. In response to increased metabolic demand or after injury, however, the adult pancreas maintains or acquires the ability to produce new cells, particularly β-cells. The precise identification of the mechanisms involved in the maintenance of β-cell mass under different conditions could offer new hints to help generating new β-cells as a cell replacement therapy for treating diabetes. Today, insulin-dependent patients rely on daily insulin injections. Transplantation of isolated islets from cadavers is problematic due to donor scarcity (about 6000 islets/kg of body weight are required [13–15]), and is only applicable to certain forms of diabetes; in addition, transplantation has met with limited success due to restricted engraftment survival [16–19]. A promising approach relies on devising unlimited *in vitro* generation of insulin-

Corresponding author: Herrera, P.L. (pedro.herrera@unige.ch)
[a] These authors contributed equally
[b] Present address: Howard Hughes Medical Institute, Whitehead Institute, and Department of Biology, Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, MA 02142, USA
[c] Present address: Howard Hughes Medical Institute, Harvard Stem Cell Institute, Department of Stem Cell and Regeneration Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138, USA

 1043-2760/$ – see front matter doi:10.1016/j.tem.2010.09.004

Table 1. Rodent models used to study pancreas regeneration. In some cases, different laboratories using the same injury approach (e.g. STZ) report different regeneration mechanisms (i.e. proliferation, neogenesis or transdifferentiation): note that in such studies, no clear assessment of the amount of β-cell loss was described and no cell tracing was performed

Model of injury	Species	Remnant β-cell mass	Regenerated β-cell mass	Markers of transitional cells	Cell tracing analysis	Mechanisms involved (or proposed)[a]	Refs
Surgical injury							
50% PX	Mouse	n.d.	100% in 4 wks		No	Proliferation?	[40]
	Mouse	n.d.	No		CldU/IdU	Proliferation	[46]
60% PX	Mouse	n.d. (∼50%)	No		*RIP-CreER*^{TM}; *Z/AP*	Proliferation	[49]
	Mouse	n.d.	68% in 2 wks		No	Proliferation	[115]
	Mouse	n.d.	73% in 1 mo		*Elast.I-CreER*^{T2}; *R26R*	No acinar transdifferentiation	[65]
	Rat	n.d.	55%		No	β-Cell hyper function	[116]
	Mouse	55%	45% in 6 wks		No	Proliferation	[117]
+sucrose	Mouse	55%	100% in 6 wks		No	Proliferation	[117]
+GLP-1	Mouse	n.d.	30% in 5 wks		No	Neogenesis/ transdifferentiation	[118]
+syngenic islets	Mouse	n.d.	70.80% in 10 d	Ngn3+ in exocrine cells Proliferating INS+	No	Proliferation/ neogenesis	[119]
90% PX	Rat	∼10%	No	Proliferating INS+	No	*Proliferation*	[120]
	Rat	∼10%	No	Proliferating ductal	No	*Neogenesis*	[121]
	Rat	∼10%	42% in 8 wks	Ductal?/Pdx1→ Pdx1+/INS+	No	Neogenesis	[29]
	Rat	∼10%	No		No	*Neogenesis*	[56]
	Rat	∼10%	No		No	*β-Cell de-differentiation*	[122]
+EX3	Rat	∼10%	50% in 4 wks	Proliferating ductal and INS+ Ductal INS+	No	Proliferation/neogenesis	[101]
+ghrelin	Rat	∼10%	No	Proliferating INS+	No	*Proliferation*	[109]
PDL	Mouse	100%	No	AMY+/INS+	No	*Transdifferentiation*	[57]
	Mouse	100%	No		*Elast.I-CreER*^{T2}; *R26R*	No acinar transdifferentiation	[65]
	Mouse	100%	200% in 1 wk	Ductal CK/Ngn3+	*Ngn3-CreERT; R26-EYFP*	Neogenesis	[30]
	Mouse	100%	No		*CAII-CreER*^{TM}; *R26R*	Ductal neogenesis/ transdifferentiation	[41]
	Mouse	100%	200% in 1 wk	No	*Hnf1β- CreER*^{TM}; *R26R*	No ductal contribution	[27]
	Rat	100%	80% in 7 d	CK19+/INS+ and CK19+/GLUT2+	No	Neogenesis	[123]
+gastrin	Rat	100%	100% in 10 d	CK7+/INS+ and CK7/GLUT2	No	Neogenesis	[104]
Cellophane wrapping	Mouse	100%	Double in 6 wks		No	*Proliferation?/ neogenesis?*	[124]
Chemical injury							
Caerulein	Mouse	100%	No	CAII+/INS+	No	*Transdifferentiation*	[60]
	Mouse	100%	No	Ductal Pdx1+	No	*Neogenesis*	[125]
	Mouse	100%	No		*Elast.I-CreER*^{T2}; *R26R*	No acinar transdifferentiation	[65]
	Rat	100%	No	Ductal Pdx1+ → Pdx1+/INS+	No	*Neogenesis*	[126]
STZ	Mouse	n.d.	No	SOM+/Pdx1+→ SOM+/Pdx1+/INS+	No	*Neogenesis*	[66]
	Mouse	n.d.	40% in 2 wks	SOM+/Pdx1+→ SOM+/Pdx1+/INS+ and GLUT2+/GLU+	No	Transdifferentiation	[67]
	Mouse	n.d.	No	AMY+/INS+	No	*Transdifferentiation*	[62]
	Mouse	n.d.	No	CK7+/Pdx1+	No	*Transdifferentiation*	[38]
	Mouse	n.d.	No	GCN+/Pdx1+ or GLUT2+ or INS+	No	*Transdifferentiation*	[31]
	Mouse	n.d.	No	Ductal Pdx1+ and Ngn3+	No	*Neogenesis*	[39]
	Rat	90%	39%	Proliferating INS+	No	Proliferation/neogenesis	[127]
+EX4 or GLP-1	Rat	n.d.	∼61% in 1 wk	Proliferating ductal and INS+	No	Neogenesis	[128]
+BTC +Activin A	Mouse	n.d.	72% in 2 mo	SOM+/Pdx1+	No	Transdifferentiation	[112]
Tungstate	Rat	n.d.	87% in 1 mo	Duct-associated INS+ Extra-islet Pdx1+	No	Neogenesis	[129]

Table 1 (*Continued*)

Model of injury	Species	Remnant β-cell mass	Regenerated β-cell mass	Markers of transitional cells	Cell tracing analysis	Mechanisms involved (or proposed)[a]	Refs
+EX4	Mouse	n.d.	No	Pdx1+/SOM+ CK7/Pdx1+	No	*Neogenesis*	[38]
+Ela-INGAP	Mouse	n.d.	No		No	*Neogenesis*	[95]
+RIP-IGF-1	Mouse	30%	50% in 3 mo	Proliferating INS+	No	Proliferation	[106]
+BTC adeno	Mouse	10%	50% in 4 wks	Proliferating CK+ and Pdx1+	No	Proliferation/neogenesis	[111]
+Ngn3/Pdx1/ MafA adeno	Mouse	n.d.	100% in 6 wks		*Cpa1-CreER^{T2}; R26R*	Transdifferentiation	[63]
+ghrelin	Rat	n.d.	No	Proliferating INS+	No	*Proliferation*	[108]
+FGF-7	Rat	n.d.	No	Proliferating ductal and INS+	No	*Proliferation/neogenesis*	[114]
Alloxan	Rat	n.d.	No	Proliferating ductal and INS+	No	*Proliferation/neogenesis*	[130]
	Rat	n.d.	No	Ductal INS+	No	*Neogenesis*	[131]
+EGF +gastrin	Mouse	n.d.	No	Ductal CK+/INS+	No	*Neogenesis*	[110]
+HB-EGF	Mouse	n.d.	No	Ductal CK+/INS+	No	*Neogenesis*	[113]
Genetic models							
Pdx1-tTA; TetO-Pdx1	Mouse	n.d.	No	Ductal Pdx1+/INS- and Pdx1+/INS+ GCN+/INS+→INS+	No	*Neogenesis/ transdifferentiation*	[33]
Kir6.2 G132S	Mouse	5.50%	70% in 5 mo	DBA+/INS+→ Pdx1-/INS+ SOM+/INS+→INS+	No	Neogenesis	[34]
RIP-Tgfα	Mouse	n.d.	No	AMY+/ductal CK+→ ductal CK+/Pdx1+	No	*Neogenesis/ transdifferentiation*	[61]
INS-Ifnγ	Mouse	n.d.	No	Proliferating INS+ and ductal INS+	No	*Proliferation/neogenesis*	[85]
	Mouse	n.d.	No	AMY+/CAII+ AMY+/INS+	No	*Transdifferentiation*	[59]
	Mouse	n.d.	No	Proliferating ductal	No	*Neogenesis*	[132]
	Mouse	n.d.	No	Ductal Pdx1+	No	*Neogenesis*	[133]
+STZ	Mouse	n.d.	No	AMY+/INS+	No	*Transdifferentiation*	[58]
RIP-Tnfα	Mouse	100%	No	Type II CK+/INS+	No	-	[84]
RIP-MycER^{TM}	Mouse	n.d.	n.d.	Proliferating DBA+ and INS+	No	*Proliferation/neogenesis*	[48]
INS-NfsB	Zebrafish	n.d.	No	Proliferating INS+	*INS-Kaede*	Proliferation and Neogenesis or transdifferentiation	[86]
RIP-rtTA; Tet-DTA	Mouse	~30%	100% in 8 mo	Proliferating INS+	*RIP-CreER^{TM}; Z/AP*	Proliferation	[50]
RIP-DTR (HB-EGF)	Mouse	0.10%	1.2% in 1 mo	INS+/GLU+	*RIP-CreER^{TM}; R26YFP*	α-Cell to β-cell transdifferentiation	[22]
			~10% in 10 mo	INS+/GLU+	*GlurtTA; TetO-Cre; R26YFP*		

[a]Regeneration mechanisms indicated in *italics* relate to experimental models in which no regeneration was detected and no cell tracing was performed, and in which the degree of β-cell loss was often not determined, but a regeneration process was nevertheless proposed by the authors. Grey rows highlight reports in which cell lineage-tracing experiments were described.Abbreviations: BTC: betacellulin; EX3: exendin3; EX4: exendin4/exenatide; GLP-1: glucagon-like peptide1; mo: months; n.d.: not determined; No: regeneration not observed; PDL: pancreatic duct ligation; PX: pancreatectomy; STZ: streptozotocin; wk(s): weeks.

producing cells derived from embryonic stem (ES) cells or, even more interestingly, from patient-derived induced pluripotent stem (iPS) cells [20,21]. Very recently, however, in view of new experimental evidence showing that adult differentiated pancreatic cells can reprogram and change their phenotype [22], exploration of the intrinsic spontaneous capacity of the adult pancreas to regenerate β-cells, in particular from heterologous origins, has acquired a new dimension as a route to the development of therapeutic treatments for diabetes.

This review will focus on β-cell regeneration and its diverse mechanisms. In fact, exploiting the intrinsic capacity of the adult pancreas to produce new β-cells endogenously is probably the most promising way to develop cell replacement therapies to treat the forms of diabetes that result from massive β-cell loss. Nevertheless, a prerequisite for such an achievement will be to uncover the immunological basis of the pathogenesis of the disease.

Induction of pancreas injury: models of regeneration

Pancreas regeneration has been studied for more than 30 years using a variety of rodent models which partially mimic either T1D (type 1 diabetes, also called juvenile or insulin-dependent diabetes mellitus) or T2D (type 2 diabetes, non-insulin-dependent diabetes mellitus), essentially by reducing the β-cell mass and therefore impairing glucose homeostasis (Table 1) (for a comprehensive review with a historical perspective see [23]).

Surgical procedures trigger acute pancreatitis. Typically, subdiabetogenic partial pancreatectomy (PX) (i.e. resection of 60–70% of the pancreas) and diabetogenic partial PX (90% resection) imply the loss of a substantial proportion of pancreatic cells, including β-cells, and can thus trigger diabetes. Other surgical approaches such as cellophane wrapping and pancreatic main duct ligation (PDL), or the administration of the drug caerulein, are protocols that trigger acute pancreatitis, as does PX, but do not involve β-cell mass reduction. For this reason these latter are, *sensu stricto*, models of pancreas tissue remodeling rather than models of pancreas regeneration, but in which interestingly there is *generation* of new β-cells. After PX, on the contrary, there is a dramatic reduction of the β-cell mass, and this is followed by a process of reconstitution or replacement (i.e. *regeneration*) of new β-cells.

Diabetes and β-cell regeneration are also observed in models of chemically- or genetically-induced β-cell destruction (Box 1). Considering the different approaches and observations that have been reported it now appears that the extent and mechanism of β-cell mass increase could be different and unique to each injury model (Table 1). Three principal regeneration processes have been described or proposed, namely: (i) neogenesis – undifferentiated precursor or progenitor cell differentiation to islet cells, (ii) replication of the remaining intact β-cells, and, more recently, (iii) transdifferentiation of pancreatic fully differentiated cells – conversion of one cell type into another (non-β cells to β-cells) by reprogramming (Figure 1).

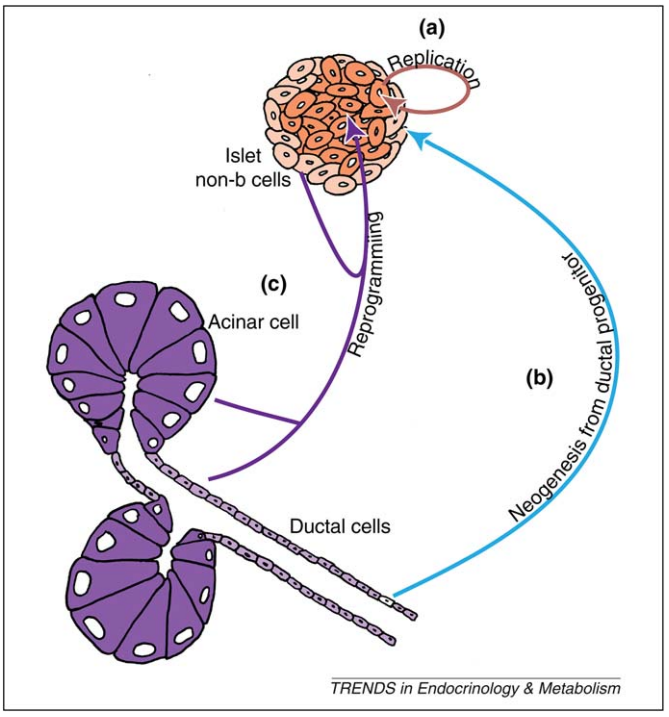

Figure 1. Cellular pathways to regeneration. At least in theory, three main processes can give rise to new β-cells: **(a)** replication of remaining β-cells, **(b)** differentiation of undifferentiated precursors located in ducts or elsewhere, and **(c)** transdifferentiation (direct reprogramming) of fully differentiated pancreatic ductal, acinar or islet non-β-cells.

Here we review the literature relating to these three processes. Papers involving cell lineage analyses will be particularly highlighted. Irrespective of the injury-and-regeneration model studied, it must be stressed that marker coexpression in single cells is not a proof *per se* of an ontogenic (i.e. mother-to-daughter) relationship between different cell types. Only the appropriate cell lineage-tracing studies allow the origin of regenerated cells to be established, thereby identifying the exact regenerative processes involved (i.e. neogenesis, β-cell replication or transdifferentiation).

Neogenesis after damage: recapitulating pancreatic ontogeny? The controversial involvement of pancreatic duct cells in β-cell regeneration

The existence of intra-islet adult stem cells was debated in the past; in particular, cells expressing the filament nestin were proposed to be such islet progenitors, yet experimental evidence did not support this possibility [24,25]. Even so, the existence of an undefined multipotent adult pancreatic stem/progenitor cell, in particular of ductal origin, is a matter of controversy, with contradictory results provided by cell lineage-tracing analyses [26–28]. It has been proposed the ductal cells represent a pool of homogeneous cells from which few dedifferentiate into progenitor cells or transdifferentiate; alternatively, ducts can contain rare persistent distinct undifferentiated progenitor cells [26,28]. On the one hand, it has been reported that PX triggers the expression of *Pdx1*, *Neurog3* and hormones in ductal cells, increasing their proliferation. This suggests that pancreatic ducts are a potential reservoir of adult progenitor cells, and thus adult regeneration would recapitulate to some extent the generation of endocrine cells

Box 1. Total β-cell ablation

A novel powerful tool to study the intrinsic capacity of the adult pancreas to replenish β-cells is the induction of conditional β-cell ablation in adult animals without inflammation or autoimmunity, contrary to what occurs in *RIP-Tnf* [84], *RIP-Ifng* [85], non-obese diabetic (NOD) mice, or in mice treated with multiple low doses of STZ. This kind of massive β-cell ablation was achieved in zebrafish by selectively expressing the bacterial gene *NfsA* encoding nitroreductase (NTR) from an insulin promoter [86]. NTR can convert prodrugs such as metronidazole into a toxic agent, leading to β-cell apoptosis. In this experiment, new β-cells appeared with surprising rapidity, about 36 h after prodrug removal. Although the identity of the source of the regenerated β-cells could not be established, cell lineage-tracing experiments revealed a variety of mechanisms, indicative of pancreas cell plasticity, including cell proliferation and differentiation of unidentified non β-cells [86].

New evidence strongly confirms the regenerative adaptability and versatility of the adult pancreas. Our laboratory recently investigated whether adult mice can produce new β-cells after their total or near-total loss, which resembles thesituation found in the pancreas of T1D patients, but without the problem of autoimmunity [22]. We relied on the transgenic expression in mice of the human gene encoding the diphtheria toxin (DT) receptor (*HBEGF*/*DTR*) on the β-cell surface (driven by an insulin promoter; *RIP-DTR* transgene). β-Cell regeneration in this extreme situation would imply the involvement of heterologous, non-β-cell sources. The *RIP-DTR* transgene specifically allowed the ablation of more than 99% of the β-cells upon DT injection. These mice became hyperglycemic 2 to 3 days after DT administration, and needed insulin treatment to survive. With time (several months), however, treated mice showed recovery of about 10% of the normal β-cell mass, with new β-cells being derived from non-β-cells, and without involving any increase in cell proliferation rates: the majority of these new β-cells were indeed reprogrammed adult α-cells [22].

that takes place during development [29–32]. On the other hand, the direct contribution of ductal cells to the formation of new β-cells after birth or after PDL has recently been challenged [26–28].

Conditional and transient downregulation of *Pdx1* triggers diabetes within two weeks using the *Pdx1-tTA* knock-in line crossed with *TetO-Pdx1* transgenic mice [33]. After doxycyclin (DOX) removal, regeneration is induced by the re-expression of *Pdx1*, with increased cell proliferation and appearance of Pdx1+ and Pdx1+/insulin+ cells associated with ducts. In another model, mice expressing the dominant-negative form of *Kir6.2 (Kcnj11)*, a subunit of a β-cell-specific K_{ATP}-channel, become diabetic at two months of age but, intriguingly, partially recover their β-cell mass by six months. The presence of islet cells coexpressing DBA lectin (a ductal cell marker), amylase and hormones led the authors to suggest that ductal cells could be involved in this process [34].

Administration of cytotoxic agents, such as streptozotocin (STZ) or alloxan, is another way to decrease β-cell mass (Box 2). These molecules enter β-cells through the glucose transporter type 2 (Glut2) and trigger either DNA damage (STZ, alkylating agent) or the production of reactive oxygen species (alloxan), leading to β-cell death (extensively reviewed in [35]). Unfortunately, however, Glut2 is also expressed by hepatocytes as well as by kidney and intestinal cells [36]. For this reason, STZ and alloxan are not β-cell-specific cytotoxic agents, and their use damages these extrapancreatic tissues as well, thus complicating the interpretation of the results. Different administration protocols of STZ can trigger either T2D- or T1D-like symptoms [37]. In STZ-treated hyperglycemic mice, *Neurog3* expression is upregulated; nevertheless, the same authors reported different *Neurog3* expression patterns (i.e. in islets or in ducts) in different papers [38,39]. In another work, the induced regeneration after 60% PX in mice did not appear to involve *Neurog3* re-expression [40].

The participation of cells expressing *Neurog3* in the production of new β-cells in adults appears to be dependent on the nature and extent of the pancreatic insult. For instance, with the model of PDL and subsequent tissue remodeling, *Neurog3*-expressing cells form near ducts and can give rise to new islet cells; specifically, *Neurog3* induction preceded an astonishing 40% increase in β-cell mass during the week following PDL [30]. Surprisingly, the same investigators were unable to track these cells as of ductal origin in a subsequent cell lineage-tracing analysis [27] using double-transgenic *Hnf1b-CreER; R26R* mice – which combine a conditional Cre recombinase (*CreER*) gene under *Hnf1b* control with the *R26R* Cre-activated *lacZ* reporter transgene. In fact, they found that 40% of the pancreatic ductal cells in adult mice could be labeled, but no β-cell expressed the reporter protein after PDL, suggesting that *Neurog3*+ cells were not of ductal origin [27]. Similarly, in an independent study using mucin1 *(Muc1)-CreER* mice, Mucin1+ (i.e. ductal) cells were shown to give rise to islet cells during development only, but not after birth [28]. These two studies challenged an earlier report describing the use of carbonic anhydrase 2 *(Car2)-CreER; R26R* mice in pancreatitis induced by PDL, which found that ductal cells in newborn mice can give rise to both acinar and endocrine cells [41].

Box 2. Biomolecules for β-cell regeneration

A number of molecules directly promote β-cell mass expansion and function. Both processes can occur, nevertheless, in absence of 'true' islet regeneration. Among these factors, some endogenous proteins were identified by high-throughput transcriptome and proteome screens of regenerating pancreata [87–90]. Whole pancreatic extracts after injury appear to promote β-cell mass augmentation in mice treated with STZ [91,92]. Specifically, two proteins of the Reg family, Reg3 (formerly named INGAP) and PSP/Reg, isolated from pancreatic extracts after cellophane wrapping or 90% partial PX, respectively, were shown to stimulate β-cell growth [93–96]. Recent clinical studies suggest that INGAP promotes insulin production in T1D patients and ameliorates glycemic control in T2D patients [97].

Extrapancreatic substances, such as intestinal hormones and growth factors that are involved in postprandial control of glycemia, also promote β-cell mass expansion. Glucagon-like peptide 1 (GLP-1, a post-translational cleavage product of the proglucagon peptide), for instance, is secreted upon meal ingestion by intestinal L-cells and by neurons located in the brainstem and hypothalamus. When released into the bloodstream, GLP-1 has a so-called 'incretin effect' on β-cells, and this accounts for at least 50% of the total insulin secretion. GLP-1 is nevertheless rapidly degraded by dipeptidyl peptidase 4 (DPP-4). Clinical trials have revealed that the GLP-1 agonist exenatide (or the very similar exendin-4), and the DDP-4 antagonist sitagliptin, can improve glucose-dependent insulin secretion in normal and T2D patients, and thus reduce the level of HbA_{1c} (glycated hemoglobin) [98]. In addition, GLP-1 appears to stimulate β-cell mass expansion by repressing β-cell apoptosis and by promoting β-cell proliferation and neogenesis. This was reported in rats after 90% PX and in mice made diabetic with STZ that were treated with exendin 4 [99]. In these mice, *Pdx1* and *Neurog3* expression in ducts was increased [38,99–101]. Further lineage-tracing experiments are needed to define the contribution of GLP-1 to the formation of new β-cells from progenitors, or by proliferation, or even by transdifferentiation [102].

Another interesting molecule is gastrin, a hormone secreted by gastric G-cells, which is a cholecystokinin homolog that promotes parietal HCl secretion in the stomach after food intake. Gastrin was reported to boost neogenesis of β-cells from ductal progenitors in *RIP-Tnf* mice and in rats after PDL, but without inducing β-cell proliferation [103,104]. During undernutrition, regeneration after 90% PX was moderate and was associated with lower plasma IGF-1 levels [105]. Conversely, STZ-treated mice overexpressing IGF-1 showed a higher β-cell proliferation rate [106].

The hormone ghrelin is secreted by D-cells of the gastric fundus. Ghrelin influences pancreatic endocrine and exocrine functions, and stimulates the release of growth hormone from the anterior pituitary [107]. Newborn rats made diabetic with STZ and given ghrelin for a week displayed increased β-cell proliferation and a better recovery than STZ-treated controls [108]. Similar observations were made in juvenile rats after 90% PX [109].

Other growth factors also promote pancreatic regeneration. Betacellulin is a member of the epidermal growth factor (EGF) family and was reported to improve β-cell mass and function in mice after administration of STZ and in rats after 90% PX [110,111]. When administered to STZ-treated neonates together with activin A (TGF-β family), β-cell mass growth was potentiated [112]. This increase in β-cell mass was associated with proliferation of ductal, β- and δ-cells, and with the appearance of cells coexpressing somatostatin and insulin, or somatostatin and Pdx1. Another EGF-like molecule, HB-EGF, also improves β-cell mass recovery by stimulating ductal β-cell neogenesis and β-cell proliferation [113]. Similar observations were made in STZ-treated newborn rats that received FGF-7 (fibroblast growth factor family) [114].

Very recently, observations made by lineage-tracing Sox9+ cells (Sox9 is an early pancreas progenitor marker, as is Pdx1) with high efficiency (70–80% of ductal cells labeled), largely correlate with those obtained using *Hnf1b-CreER; R26R* mice [27]: after PDL, Sox9+ ductal cells give rise to *Neurog*+ cells, but do not contribute to islets [42].

In conclusion, whether there is a direct contribution of ductal cells to the formation of new β-cells in adult mice, be they differentiated epithelial cells or undifferentiated precursors, remains elusive, even if negative evidence is accumulating in most recent reports [43–45]. What could explain the discrepancy? The contradictory observations might be explained, at least in part, by (i) heterogeneity in the ductal cell population – duct cell labeling was mosaic in most lineage-tracing studies (between 60 to 20% of duct cells remained unlabeled in different works), or by (ii) transgene specificity or other technical issues. What is the tenet regarding the role of pancreatic ducts in β-cell regeneration? Although the issue remains unresolved at this time, it now clearly appears (and future studies should confirm this view) that there is a high degree of versatility and cell plasticity in the adult pancreas, such that the nature and extent of the lesion inflicted to this organ probably determines the type of healing or regenerative response.

Replication of remaining β-cells

Several studies describe increased β-cell mitotic indexes in injured pancreata, and this suggests that β-cell regeneration can result from the proliferation of pre-existing β-cells. This was shown, for instance, using nucleotide-analog-dependent lineage tracing after 50% PX in mice [46]. In another study, transient forced expression of *Myc* in β-cells, which initially leads to massive apoptosis [47], subsequently resulted in a higher mitotic index in remaining β-cells and regeneration [48]; in this investigation, however, no cell lineage-tracing was performed.

In 60% of PX mice, or in transgenic models in which an important degree of β-cell death can be induced, such as the insulin (*Ins2-rtTA*)-*rtTA; TetO-DTA* mice, cell lineage-tracing analyses showed surviving β-cells to be the precursors of new β-cells [49,50]. Briefly, mice were given DOX for several weeks, including the gestational period, to trigger the transgenic expression in β-cells of DT-A (i.e. the active subunit of diphtheria toxin, DT, a blocker of protein synthesis) [51]. In these animals about 70–80% of the β-cell mass was destroyed and they became diabetic. After DOX removal β-cell mass was fully replenished by new β-cells produced by the replication of remaining β-cells, as shown by β-cell tracing analysis and BrdU incorporation [50].

In rodent β-cells, cell cycling is driven by CyclinD1/D2-Cdk4 activity and is repressed by the Cdk-inhibitor $p16^{INK4a}$ [44,45]. After STZ treatment, mice expressing an active (i.e. p16-independent) $Cdk4^{R}[24]^{C}$ mutant kinase very rapidly showed improved β-cell regeneration, and this correlated with an increased number of proliferating cells in ducts and islets as compared with untreated mice [51], but no lineage tracing was done. In wild-type mice, the Cdk inhibitor $p16^{INK4a}$ accumulates with age and is associated with the observed decline in aged animals of the regenerative potential after STZ treatment [52,53]. Similarly, the expression of another Cdk inhibitor, $p21^{CIP}$, represses β-cell replication, but interestingly, STZ-treated mice overexpressing $p21^{CIP}$ showed enhanced expression of developmental genes, such as *Pdx1* and *Neurog3* [54].

In conclusion, although β-cell replication capacity declines with age [12,52,53], it appears to be the main compensatory mechanism for β-cell mass maintenance in different injury models in which significant proportion (20–30%) of β-cells persist [43–45], or in situations of increased metabolic demand, with increased resistance to insulin, such as during pregnancy (compensation failure results in gestational diabetes in some 3–10% of human pregnancies). In this respect it was recently shown that lactogenic hormones stimulate serotonin production by β-cells in pregnant mice, and this can drive β-cell proliferation [23]. These findings will probably lead to new discoveries of how β-cells can be directed to re-enter the cell cycle.

Reprogramming pancreatic cells into β-cells

Fate switching of adult cells, or transdifferentiation/reprogramming of terminally differentiated cells to another phenotype, is, in principle, a rare event that can take place either via direct conversion, or indirectly through a transient intermediate de-differentiated stage – which does not systematically involve the passage through a transitional pluripotent stage [55]. In different mouse injury models the detection of transitional cells (i.e. cells expressing multiple markers) suggests that exocrine cells can transdifferentiate directly into β-cells – ductal cells after 90% PX [56], acinar cells after PDL [57], and acinar and ductal cells in *human insulin promoter-Ifng* mice in which γ-interferon expression is driven from the insulin gene promoter [58] (Table 1). The coexpression of insulin and amylase has led to the hypothesis that acinar cells could represent progenitors to β-cells during regeneration in these different models [57,59–62]. In fact, two studies using genetic lineage-tracing approaches have so far demonstrated direct reprogramming of adult acinar cells, either to β-cells or to adipocytes, by ectopic β-cell-specific transcription factor expression or after inactivation of *Myc*, respectively [63,64].

In elastase I (*Ela1*)-*CreERT2; R26R* mice, adult acinar cells can be conditionally labeled with moderate efficiency, but the proportion of labeled cells is high in few pancreatic lobes [65]. Desai and colleagues showed with these transgenics that there is no acinar-to-β-cell reprogramming after 70% PX or in PDL- or caerulein-induced pancreatitis. In a more recent study, however, using the same transgenic acinar lineage-tracing system, Zhou and coworkers have shown that overexpression of β-cell lineage-specific genes (namely *Neurog3*, *Pdx1* and *MafA*) in adult acinar cells, from recombinant adenovirus injected into the pancreatic parenchyma, forces them to acquire the β-cell phenotype [63]. Together these results suggest that acinar cells are plastic enough to reprogram when driven by transcription factor activation or inactivation, but do not spontaneously reprogram during inflammation or after β-cell mass reduction.

Islet endocrine non-β-cells could also represent a potential source of new β-cells. For instance, cells coexpressing different hormones have been described after STZ treatment or cellophane wrapping [34,58,66–68]. More precise-

ly, some investigators suggested the potential involvement of α-cells in β-cell regeneration after observing cells coexpressing glucagon and insulin in several pancreatic regeneration models. Another interesting observation is that diabetic patients have an increased α-cell mass [69,70]; similarly, α-cell proliferation was increased in STZ-treated rats, and α-cells expressed β-cell markers such as Pdx1, Glut2 (Slc2a2) and insulin [31,66,71]. In the same way, β-cell ablation induced by DOX in *Ins2-rtTA; TetO-DTA* mice was followed by the appearance of a very small number of glucagon+/insulin+ bihormonal cells, although these cells were not lineage-traced and their contribution to the formation of new β-cells was not explored [50]. *In vitro*, the α-cell line αTC1.6 expresses various β-cell markers when cultured in the presence of betacellulin [72].

Lineage-tracing studies, performed in healthy mice after the initial reports on bihormonal cells were published, revealed that adult α- and β-cells share a common precursor but belong to separate developmental lineages [73]. However, Collombat and colleagues recently reported that ectopic expression of the β-cell-specific transcription factor *Pax4* in mouse embryonic α-cells triggers their conversion into β-cells [74]. Conversely, downregulation of *Pdx1* in β-cells triggers the expression of glucagon, thus leading to the accumulation of cells coexpressing insulin and glucagon [33,75,76]; similarly, upregulation of *Arx* in embryonic β-cells pushes them towards the acquisition of the α-cell phenotype [77].

In summary, recent observations reveal that the directed expression of α-cell-specific transcription factors in developing β-cells [77], or of β-cell lineage transcription factors in either developing α-cells [74] or in adult differentiated acinar cells [63], is sufficient to trigger their reprogramming in non-regenerating conditions, and illustrates the plasticity of pancreatic cells.

Very recent evidence now shows that regenerating conditions could be sufficient to drive the spontaneous conversion of adult pancreatic cells. The heterologous formation of new β-cells in adulthood was recently shown in *RIP-DTR* mice after the near total ablation of β-cells, with an α-cell-specific inducible lineage-tracing experiment performed during regeneration [22] (Box 1). After near-total loss of β-cells, about 5–10% of the α-cells in these mice spontaneously and rapidly reprogram to produce insulin and other β-cell proteins, including Pdx1, Nkx6.1 or Glut2, and therefore the majority of the regenerated β-cells are indeed reprogrammed α-cells. Direct α-to-β reprogramming was further confirmed in this study by double ablation of β- and α-cells; this prevented the appearance of glucagon+/insulin+ bihormonal cells. The molecular mechanisms governing the α- to β-cell conversion have not yet been deciphered; systemic or local factors, or both (Box 2), might be involved in this newly-observed process.

Perspectives

The number of articles reporting the capacity of the adult pancreas to generate new cells after damage is growing. Evidence from the somewhat abundant literature suggests that regeneration must involve different mechanisms that are directly related to (i) the type of injury, and (ii) the amount or degree of β-cell loss (Figure 2; summarized in Table 1).

We are entering an exciting period in which we should learn how to exploit the regenerative power of the pancreas. Acinar and α-cells have been shown so far to acquire the β-cell phenotype upon genetic modification or after near-total β-cell loss. This versatility could be used to trigger cell reprogramming in diabetes, but more mechanistic information will be required before the endogenous capacity of the pancreas to generate new β-cells can be exploited. For example, diverse molecules including circulating gut hormones and local growth factors, or cytokines in autoimmune or inflammatory conditions, can modulate regeneration [78,79] (Box 2).

In humans, as in rodents, pancreas regeneration appears to be modulated by age and disease. One-month-old infants afflicted with 'permanent hyperinsulinemic hypoglycaemia in infancy' can only be treated by

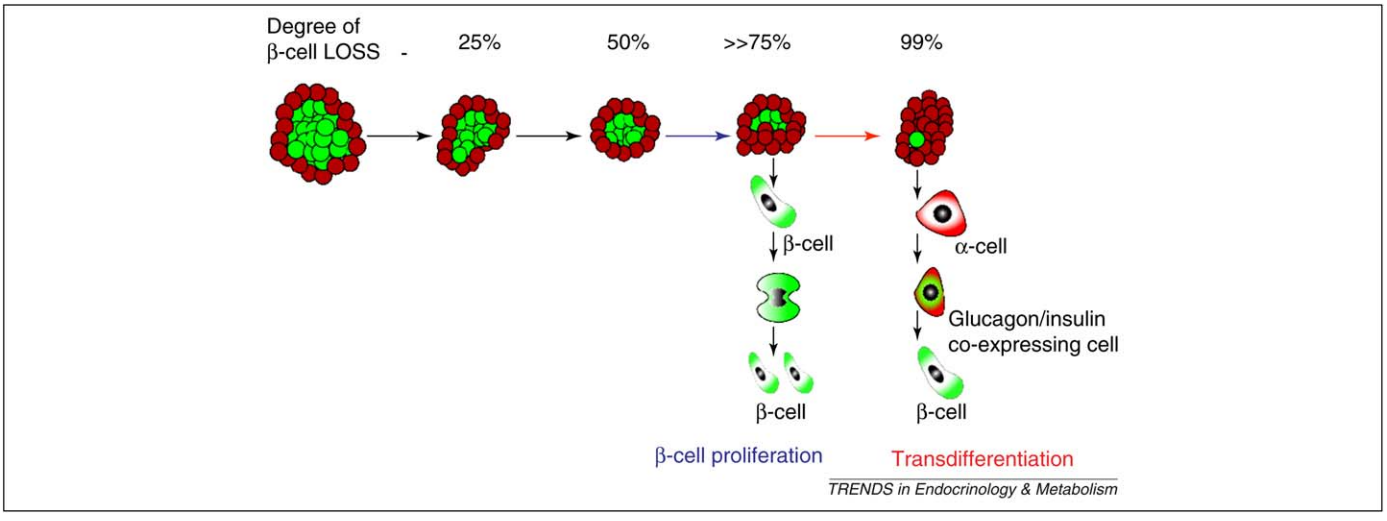

Figure 2. The amount of β-cell loss and the type of injury determine the regeneration mechanism. The contribution of procedures other than β-cell proliferation is proportional to the degree of β-cell destruction, such that the less β-cells remain, the more relevant are processes such as direct reprogramming of other pancreatic cell types to β-cells. Only a profound decrease of the β-cell mass triggers β-cell regeneration – for example, 50% β-cell ablation in *RIP-DTR* female mice is not followed by a measurable increase in the β-cell mass under normal conditions (i.e. without inflammation and on a standard diet) [22]. The heterologous formation of new β-cells is only observed when almost no β-cells are left [22]. Green cells, β-cells; red cells, α-cells.

removal of most of the pancreas (90–95% PX), and this results in iatrogenic diabetes. Over a follow-up period of almost 6 years in one study, 12 out of 22 children having undergone >90% PX recovered glycemic control, meaning that they had regenerated significant β-cell mass [80]. On the contrary, aged patients (50 years on average) undergoing 50% PX as treatment for adenocarcinoma or chronic pancreatitis did not show any measurable regeneration after a period of almost 2 years. Whether this is an age-related phenomenon or whether 50% PX is not enough to stimulate regeneration, or 2 years is insufficient, remains unclear [81]. Nevertheless, there is evidence suggesting that regeneration by increased β-cell replication is not blocked in aged individuals with T1D [82]. In another study involving a cohort of recent-onset T1D patients who died of diabetic ketoacidosis there were no signs of increased β-cell replication [83].

These combined observations in humans correlate with our own observations made in mice after total β-cell ablation showing that the adult pancreas can generate new β-cells from heterologous sources [22]. We can thus reasonably speculate that the development of a sustainable regenerative therapy for treating T1D will require our mastering the ability to control the immune system; in this situation any inherent regenerative capacity of the adult pancreas would become apparent and therefore exploitable in the development of new and powerful treatments.

Acknowledgements

We are grateful to F. Thorel, S. Chera and D. Baronnier for critically reading the manuscript, and to S. Chera for artwork. We thank S. Tranguch for excellent editorial support. We were unable to cite all the extensive literature on pancreas regeneration. C.B. and R.D. are recipients of grants (postdoctoral fellowship awards) from the Swiss National Science Foundation. P.L.H. is a recipient of grants from the Juvenile Diabetes Research Foundation, the National Institutes of Health (NIH)/National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (Beta Cell Biology Consortium), the Swiss National Science Foundation (National Centre of Competence in Research 'Frontiers-in-Genetics' and NRP63), and the European Union (FP6 and FP7).

References

1 Grondin, G. *et al.* (1997) Models of exocrine pancreatic pathologies: a microscopical point of view. *Microsc. Res. Tech.* 36, 324–336
2 Prado, C.L. *et al.* (2004) Ghrelin cells replace insulin-producing beta cells in two mouse models of pancreas development. *Proc. Natl. Acad. Sci. U. S. A.* 101, 2924–2929
3 Reimer, M.K. *et al.* (2003) Dose-dependent inhibition by ghrelin of insulin secretion in the mouse. *Endocrinology* 144, 916–921
4 Granata, R. *et al.* (2010) Unraveling the role of the ghrelin gene peptides in the endocrine pancreas. *J. Mol. Endocrinol.* 45, 107–118
5 Stanger, B.Z. *et al.* (2007) Organ size is limited by the number of embryonic progenitor cells in the pancreas but not the liver. *Nature* 445, 886–891
6 Desgraz, R. and Herrera, P.L. (2009) Pancreatic neurogenin 3-expressing cells are unipotent islet precursors. *Development* 136, 3567–3574
7 Zhou, Q. *et al.* (2007) A multipotent progenitor domain guides pancreatic organogenesis. *Dev. Cell* 13, 103–114
8 Johansson, K.A. *et al.* (2007) Temporal control of neurogenin3 activity in pancreas progenitors reveals competence windows for the generation of different endocrine cell types. *Dev. Cell* 12, 457–465
9 Holemans, K. *et al.* (2003) Lifetime consequences of abnormal fetal pancreatic development. *J. Physiol.* 547, 11–20
10 Xita, N. and Tsatsoulis, A. (2010) Fetal origins of the metabolic syndrome. *Ann. N. Y. Acad. Sci.* 1205, 148–155
11 Ng *et al.* (2010) Chronic high-fat diet in fathers programs beta-cell dysfunciton in female rat offspring. *Nature* 467, 963–966
12 Teta, M. *et al.* (2005) Very slow turnover of beta-cells in aged adult mice. *Diabetes* 54, 2557–2567
13 Robertson, R.P. (2010) Update on transplanting beta cells for reversing type 1 diabetes. *Endocrinol. Metab. Clin. North Am.* 39, 655–667
14 Robertson, R.P. (2010) Islet transplantation a decade later and strategies for filling a half-full glass. *Diabetes* 59, 1285–1291
15 Ridgway, D. *et al.* (2010) Preservation of the donor pancreas for whole pancreas and islet transplantation. *Clin. Transplant.* 24, 1–19
16 Shapiro, A.M. *et al.* (2006) International trial of the Edmonton protocol for islet transplantation. *N. Engl. J. Med.* 355, 1318–1330
17 Toso, C. *et al.* (2006) Sequential kidney/islet transplantation: efficacy and safety assessment of a steroid-free immunosuppression protocol. *Am. J. Transplant.* 6, 1049–1058
18 Cure, P. *et al.* (2008) Improved metabolic control and quality of life in seven patients with type 1 diabetes following islet after kidney transplantation. *Transplantation* 85, 801–812
19 Deng, S. *et al.* (2009) Islet alone versus islet after kidney transplantation: metabolic outcomes and islet graft survival. *Transplantation* 88, 820–825
20 Rood, P.P. *et al.* (2006) Facilitating physiologic self-regeneration: a step beyond islet cell replacement. *Pharm. Res.* 23, 227–242
21 Tateishi, K. *et al.* (2008) Generation of insulin-secreting islet-like clusters from human skin fibroblasts. *J. Biol. Chem.* 283, 31601–31607
22 Thorel, F. *et al.* (2010) Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. *Nature* 464, 1149–1154
23 Granger, A. and Kushner, J.A. (2009) Cellular origins of beta-cell regeneration: a legacy view of historical controversies. *J. Intern. Med.* 266, 325–338
24 Esni, F. *et al.* (2004) Origin of exocrine pancreatic cells from nestin-positive precursors in developing mouse pancreas. *Mechanisms of Development* 121, 15–25
25 Delacour, A. *et al.* (2004) Nestin expression in pancreatic exocrine cell lineages. *Mechanisms of Development* 121, 3–14
26 Bonner-Weir, S. and Weir, G.C. (2005) New sources of pancreatic beta-cells. *Nat. biotechnol.* 23, 857–861
27 Solar, M. *et al.* (2009) Pancreatic exocrine duct cells give rise to insulin-producing beta cells during embryogenesis but not after birth. *Dev. Cell* 17, 849–860
28 Kopinke, D. and Murtaugh, L.C. (2010) Exocrine-to-endocrine differentiation is detectable only prior to birth in the uninjured mouse pancreas. *BMC Dev. Biol.* 10, 38
29 Sharma, A. *et al.* (1999) The homeodomain protein IDX-1 increases after an early burst of proliferation during pancreatic regeneration. *Diabetes* 48, 507–513
30 Xu, X. *et al.* (2008) Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell* 132, 197–207
31 Thyssen, S. *et al.* (2006) Ontogeny of regeneration of beta-cells in the neonatal rat after treatment with streptozotocin. *Endocrinology* 147, 2346–2356
32 Bonner-Weir, S. *et al.* (2004) The pancreatic ductal epithelium serves as a potential pool of progenitor cells. *Pediat. Diabetes* 5 (Suppl. 2), 16–22
33 Holland, A.M. *et al.* (2005) Conditional expression demonstrates the role of the homeodomain transcription factor Pdx1 in maintenance and regeneration of beta-cells in the adult pancreas. *Diabetes* 54, 2586–2595
34 Oyama, K. *et al.* (2006) Spontaneous recovery from hyperglycemia by regeneration of pancreatic beta-cells in Kir6.2G132S transgenic mice. *Diabetes* 55, 1930–1938
35 Lenzen, S. (2008) The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia* 51, 216–226
36 Thorens, B. (1996) Glucose transporters in the regulation of intestinal, renal, and liver glucose fluxes. *Am. J. Physiol.* 270, G541–553
37 Like, A.A. and Rossini, A.A. (1976) Streptozotocin-induced pancreatic insulitis: new model of diabetes mellitus. *Science* 193, 415–417
38 Kodama, S. *et al.* (2005) Enhanced expression of PDX-1 and Ngn3 by exendin-4 during beta cell regeneration in STZ-treated mice. *Biochem. Biophys. Res. Commun.* 327, 1170–1178
39 Kodama, M. *et al.* (2009) Embryonic stem cell transplantation correlates with endogenous neurogenin 3 expression and pancreas

regeneration in streptozotocin-injured mice. *J. Histochem. Cytochem.* 57, 1149–1158
40 Lee, C.S. *et al.* (2006) Regeneration of pancreatic islets after partial pancreatectomy in mice does not involve the reactivation of neurogenin-3. *Diabetes* 55, 269–272
41 Inada, A. *et al.* (2008) Carbonic anhydrase II-positive pancreatic cells are progenitors for both endocrine and exocrine pancreas after birth. *Proc. Natl. Acad. Sci. U. S. A.* 105, 19915–19919
42 Sander, M. Personal communication
43 Zaret, K. (2004) Regenerative medicine: self-help for insulin cells. *Nature* 429, 30–31
44 Zhang, X. *et al.* (2005) Overexpression of cyclin D1 in pancreatic beta-cells *in vivo* results in islet hyperplasia without hypoglycemia. *Diabetes* 54, 712–719
45 Marzo, N. *et al.* (2004) Pancreatic islets from cyclin-dependent kinase 4/R24C (Cdk4) knockin mice have significantly increased beta cell mass and are physiologically functional, indicating that Cdk4 is a potential target for pancreatic beta cell mass regeneration in type 1 diabetes. *Diabetologia* 47, 686–694
46 Teta, M. *et al.* (2007) Growth and regeneration of adult beta cells does not involve specialized progenitors. *Dev. Cell* 12, 817–826
47 Pelengaris, S. *et al.* (2002) Suppression of Myc-induced apoptosis in beta cells exposes multiple oncogenic properties of Myc and triggers carcinogenic progression. *Cell* 109, 321–334
48 Cano, D.A. *et al.* (2008) Regulated beta-cell regeneration in the adult mouse pancreas. *Diabetes* 57, 958–966
49 Dor, Y. *et al.* (2004) Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 429, 41–46
50 Nir, T. *et al.* (2007) Recovery from diabetes in mice by beta cell regeneration. *J. Clin. Invest.* 117, 2553–2561
51 Lee, J.H. *et al.* (2010) Cdk4 regulates recruitment of quiescent beta-cells and ductal epithelial progenitors to reconstitute beta-cell mass. *PloS One* 5, e8653
52 Wang, R.N. *et al.* (1996) Beta-cell growth in adolescent and adult rats treated with streptozotocin during the neonatal period. *Diabetologia* 39, 548–557
53 Krishnamurthy, J. *et al.* (2006) p16INK4a induces an age-dependent decline in islet regenerative potential. *Nature* 443, 453–457
54 Yang, J. *et al.* (2009) P21cip-overexpression in the mouse beta cells leads to the improved recovery from streptozotocin-induced diabetes. *PloS One* 4, e8344
55 Zhou, Q. and Melton, D.A. (2008) Extreme makeover: converting one cell into another. *Cell Stem Cell* 3, 382–388
56 Finegood, D.T. *et al.* (1999) Prior streptozotocin treatment does not inhibit pancreas regeneration after 90% pancreatectomy in rats. *Am. J. Physiol.* 276, E822–827
57 Bertelli, E. and Bendayan, M. (1997) Intermediate endocrine-acinar pancreatic cells in duct ligation conditions. *Am. J. Physiol.* 273, C1641–1649
58 Gu, D. *et al.* (1997) Endocrine/exocrine intermediate cells in streptozotocin-treated Ins-IFN-gamma transgenic mice. *Pancreas* 15, 246–250
59 Gu, D. *et al.* (1994) Transitional cells in the regenerating pancreas. *Development* 120, 1873–1881
60 Walker, N.I. *et al.* (1992) Ultrastructure of the rat pancreas after experimental duct ligation. II. Duct and stromal cell proliferation, differentiation, and deletion. *Pancreas* 7, 420–434
61 Song, S.Y. *et al.* (1999) Expansion of Pdx1-expressing pancreatic epithelium and islet neogenesis in transgenic mice overexpressing transforming growth factor alpha. *Gastroenterology* 117, 1416–1426
62 Aughsteen, A.A. (2002) Immunofluorescence and electron-microscopic observations of intermediate cells in the pancreas of mice, rats and humans. *Cells Tissues Organs* 170, 21–28
63 Zhou, Q. *et al.* (2008) *In vivo* reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature* 455, 627–632
64 Bonal, C. *et al.* (2009) Pancreatic inactivation of c-Myc decreases acinar mass and transdifferentiates acinar cells into adipocytes in mice. *Gastroenterology* 136, 309–319 e309
65 Desai, B.M. *et al.* (2007) Preexisting pancreatic acinar cells contribute to acinar cell, but not islet beta cell, regeneration. *J. Clin. Invest.* 117, 971–977
66 Fernandes, A. *et al.* (1997) Differentiation of new insulin-producing cells is induced by injury in adult pancreatic islets. *Endocrinology* 138, 1750–1762
67 Guz, Y. *et al.* (2001) Regeneration of pancreatic beta cells from intra-islet precursor cells in an experimental model of diabetes. *Endocrinology* 142, 4956–4968
68 Rosenberg, L. *et al.* (1989) The effect of cellophane wrapping of the pancreas in the Syrian golden hamster: autoradiographic observations. *Pancreas* 4, 31–37
69 O'Reilly, L.A. *et al.* (1997) Alpha-cell neogenesis in an animal model of IDDM. *Diabetes* 46, 599–606
70 Guardado-Mendoza, R. *et al.* (2009) Pancreatic islet amyloidosis, beta-cell apoptosis, and alpha-cell proliferation are determinants of islet remodeling in type-2 diabetic baboons. *Proc. Natl. Acad. Sci. U. S. A.* 106, 13992–13997
71 Kim, B.M. *et al.* (2001) Clusterin expression during regeneration of pancreatic islet cells in streptozotocin-induced diabetic rats. *Diabetologia* 44, 2192–2202
72 Watada, H. *et al.* (1996) PDX-1 induces insulin and glucokinase gene expression in alphaTC1 clone 6 cells in the presence of betacellulin. *Diabetes* 45, 1826–1831
73 Herrera, P.L. (2000) Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. *Development* 127, 2317–2322
74 Collombat, P. *et al.* (2009) The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into alpha and subsequently beta cells. *Cell* 138, 449–462
75 Ahlgren, U. *et al.* (1998) Beta-cell-specific inactivation of the mouse *Ipf1/Pdx1* gene results in loss of the beta-cell phenotype and maturity onset diabetes. *Genes Dev.* 12, 1763–1768
76 Thomas, M.K. *et al.* (2001) Development of diabetes mellitus in aging transgenic mice following suppression of pancreatic homeoprotein IDX-1. *J. Clin. Invest.* 108, 319–329
77 Collombat, P. *et al.* (2007) Embryonic endocrine pancreas and mature beta cells acquire alpha and PP cell phenotypes upon Arx misexpression. *J. Clin. Invest.* 117, 961–970
78 Bouwens, L. and Rooman, I. (2005) Regulation of pancreatic beta-cell mass. *Physiol. Rev.* 85, 1255–1270
79 Akirav, E. *et al.* (2008) Beta-cell mass and type 1 diabetes: going, going, gone? *Diabetes* 57, 2883–2888
80 Berrocal, T. *et al.* (2005) Pancreatic regeneration after near-total pancreatectomy in children with nesidioblastosis. *Pediatr. Radiol.* 35, 1066–1070
81 Menge, B.A. *et al.* (2008) Partial pancreatectomy in adult humans does not provoke beta-cell regeneration. *Diabetes* 57, 142–149
82 Meier, J.J. *et al.* (2006) Direct evidence of attempted beta cell regeneration in an 89-year-old patient with recent-onset type 1 diabetes. *Diabetologia* 49, 1838–1844
83 Butler, A.E. *et al.* (2007) Modestly increased beta cell apoptosis but no increased beta cell replication in recent-onset type 1 diabetic patients who died of diabetic ketoacidosis. *Diabetologia* 50, 2323–2331
84 Higuchi, Y. *et al.* (1992) Expression of a tumor necrosis factor alpha transgene in murine pancreatic beta cells results in severe and permanent insulitis without evolution towards diabetes. *J. Exp. Med.* 176, 1719–1731
85 Gu, D. and Sarvetnick, N. (1993) Epithelial cell proliferation and islet neogenesis in IFN-γ transgenic mice. *Development* 118, 33–46
86 Pisharath, H. *et al.* (2007) Targeted ablation of beta cells in the embryonic zebrafish pancreas using E. coli nitroreductase. *Mech. Dev.* 124, 218–229
87 Lim, H.W. *et al.* (2002) Identification of differentially expressed mRNA during pancreas regeneration of rat by mRNA differential display. *Biochem. Biophys. Res. Commun.* 299, 806–812
88 Katsuta, H. *et al.* (2005) cDNA microarray analysis after laser microdissection in proliferating islets of partially pancreatectomized mice. *Med. Mol. Morphol.* 38, 30–35
89 Shin, J.S. *et al.* (2005) Proteome analysis of rat pancreas induced by pancreatectomy. *Biochim. Biophys. Acta* 1749, 23–32
90 Yang, J. *et al.* (2005) Metabolic response of mice to a postnatal ablation of CCAAT/enhancer-binding protein alpha. *J. Biol. Chem.* 280, 38689–38699

91 Kim, Y.S. *et al.* (2003) Enhancement of mouse pancreatic regeneration and HIT-T15 cell proliferation with rat pancreatic extract. *Biochem. Biophys. Res. Commun.* 309, 528–532
92 Hardikar, A.A. and Bhonde, R.R. (1999) Modulating experimental diabetes by treatment with cytosolic extract from the regenerating pancreas. *Diabetes Res. Clin. Pract.* 46, 203–211
93 Rosenberg, L. (1998) Induction of islet cell neogenesis in the adult pancreas: the partial duct obstruction model. *Microsc. Res. Tech.* 43, 337–346
94 Terazono, K. *et al.* (1990) Expression of reg protein in rat regenerating islets and its co-localization with insulin in the beta cell secretory granules. *Diabetologia* 33, 250–252
95 Taylor-Fishwick, D.A. *et al.* (2006) Islet neogenesis associated protein transgenic mice are resistant to hyperglycemia induced by streptozotocin. *J. Endocrinol.* 190, 729–737
96 Okamoto, H. (1999) The *Reg* gene family and Reg proteins: with special attention to the regeneration of pancreatic beta-cells. *J. Hepatobiliary Pancreat. Surg.* 6, 254–262
97 Lipsett, M. *et al.* (2007) The role of islet neogenesis-associated protein (INGAP) in islet neogenesis. *Cell Biochem. Biophys.* 48, 127–137
98 Kim, W. and Egan, J.M. (2008) The role of incretins in glucose homeostasis and diabetes treatment. *Pharmacol. Rev.* 60, 470–512
99 Bulotta, A. *et al.* (2004) The role of GLP-1 in the regulation of islet cell mass. *Cell Biochem. Biophys.* 40, 65–78
100 Stoffers, D.A. (2004) The development of beta-cell mass: recent progress and potential role of GLP-1. *Horm. Metab. Res.* 36, 811–821
101 Xu, G. *et al.* (1999) Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes* 48, 2270–2276
102 Chia, C.W. and Egan, J.M. (2008) Incretin-based therapies in type 2 diabetes mellitus. *J. Clin. Endocrinol. Metab.* 93, 3703–3716
103 Wang, T.C. *et al.* (1993) Pancreatic gastrin stimulates islet differentiation of transforming growth factor alpha-induced ductular precursor cells. *J. Clin. Invest.* 92, 1349–1356
104 Rooman, I. *et al.* (2002) Gastrin stimulates beta-cell neogenesis and increases islet mass from transdifferentiated but not from normal exocrine pancreas tissue. *Diabetes* 51, 686–690
105 Fernandez, E. *et al.* (2006) Undernutrition does not alter the activation of beta-cell neogenesis and replication in adult rats after partial pancreatectomy. *Am. J. Physiol.* 291, E913–921
106 Agudo, J. *et al.* (2008) IGF-I mediates regeneration of endocrine pancreas by increasing beta cell replication through cell cycle protein modulation in mice. *Diabetologia* 51, 1862–1872
107 Wierup, N. *et al.* (2002) The ghrelin cell: a novel developmentally regulated islet cell in the human pancreas. *Regul. Pept.* 107, 63–69
108 Irako, T. *et al.* (2006) Ghrelin prevents development of diabetes at adult age in streptozotocin-treated newborn rats. *Diabetologia* 49, 1264–1273
109 Kerem, M. *et al.* (2009) Exogenous ghrelin enhances endocrine and exocrine regeneration in pancreatectomized rats. *J. Gastrointest. Surg.* 13, 775–783
110 Rooman, I. and Bouwens, L. (2004) Combined gastrin and epidermal growth factor treatment induces islet regeneration and restores normoglycaemia in C57Bl6/J mice treated with alloxan. *Diabetologia* 47, 259–265
111 Shin, S. *et al.* (2008) Remission of diabetes by beta-cell regeneration in diabetic mice treated with a recombinant adenovirus expressing betacellulin. *Mol. Ther.* 16, 854–861
112 Li, L. *et al.* (2004) Activin A and betacellulin: effect on regeneration of pancreatic beta-cells in neonatal streptozotocin-treated rats. *Diabetes* 53, 608–615
113 Kozawa, J. *et al.* (2005) Regenerative and therapeutic effects of heparin-binding epidermal growth factor-like growth factor on diabetes by gene transduction through retrograde pancreatic duct injection of adenovirus vector. *Pancreas* 31, 32–42
114 Movassat, J. and Portha, B. (2007) Early administration of keratinocyte growth factor improves {beta}-cell regeneration in rat with streptozotocin-induced diabetes. *J. Endocrinol.* 195, 333–340
115 Peshavaria, M. *et al.* (2006) Regulation of pancreatic beta-cell regeneration in the normoglycemic 60% partial-pancreatectomy mouse. *Diabetes* 55, 3289–3298
116 Liu, Y.Q. *et al.* (2000) Beta-cell adaptation in 60% pancreatectomy rats that preserves normoinsulinemia and normoglycemia. *Am. J. Physiol.* 279, E68–73
117 Leahy, J.L. *et al.* (1988) Minimal chronic hyperglycemia is a critical determinant of impaired insulin secretion after an incomplete pancreatectomy. *J. Clin. Invest.* 81, 1407–1414
118 De Leon, D.D. *et al.* (2003) Role of endogenous glucagon-like peptide-1 in islet regeneration after partial pancreatectomy. *Diabetes* 52, 365–371
119 Jung, H.S. *et al.* (2009) Enhancement of beta-cell regeneration by islet transplantation after partial pancreatectomy in mice. *Transplantation* 88, 354–359
120 Brockenbrough, J.S. *et al.* (1988) Discordance of exocrine and endocrine growth after 90% pancreatectomy in rats. *Diabetes* 37, 232–236
121 Bonner-Weir, S. *et al.* (1993) A second pathway for regeneration of adult exocrine and endocrine pancreas. A possible recapitulation of embryonic development. *Diabetes* 42, 1715–1720
122 Laybutt, D.R. *et al.* (2003) Critical reduction in beta-cell mass results in two distinct outcomes over time. Adaptation with impaired glucose tolerance or decompensated diabetes. *J. Biol. Chem.* 278, 2997–3005
123 Wang, R.N. *et al.* (1995) Duct- to islet-cell differentiation and islet growth in the pancreas of duct-ligated adult rats. *Diabetologia* 38, 1405–1411
124 Rosenberg, L. *et al.* (1983) A new approach to the induction of duct epithelial hyperplasia and nesidioblastosis by cellophane wrapping of the hamster pancreas. *J. Surg. Res.* 35, 63–72
125 Jensen, J.N. *et al.* (2005) Recapitulation of elements of embryonic development in adult mouse pancreatic regeneration. *Gastroenterology* 128, 728–741
126 Taguchi, M. *et al.* (2002) Induction of PDX-1-positive cells in the main duct during regeneration after acute necrotizing pancreatitis in rats. *J. Pathol.* 197, 638–646
127 Wang, R.N. *et al.* (1994) Beta-cell proliferation in normal and streptozotocin-treated newborn rats: site, dynamics and capacity. *Diabetologia* 37, 1088–1096
128 Tourrel, C. *et al.* (2001) Glucagon-like peptide-1 and exendin-4 stimulate beta-cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age. *Diabetes* 50, 1562–1570
129 Fernandez-Alvarez, J. *et al.* (2004) Stable and functional regeneration of pancreatic beta-cell population in nSTZ-rats treated with tungstate. *Diabetologia* 47, 470–477
130 Waguri, M. *et al.* (1997) Demonstration of two different processes of beta-cell regeneration in a new diabetic mouse model induced by selective perfusion of alloxan. *Diabetes* 46, 1281–1290
131 De Haro-Hernandez, R. *et al.* (2004) Regeneration of beta-cells and neogenesis from small ducts or acinar cells promote recovery of endocrine pancreatic function in alloxan-treated rats. *Arch. Med. Res.* 35, 114–120
132 Gu, D. *et al.* (1995) Treatment of IFN-gamma transgenic mice with anti-IFN-gamma reveals the remodeling capacity of the adult pancreas. *Diabetes* 44, 1161–1164
133 Kritzik, M.R. *et al.* (1999) PDX-1 and Msx-2 expression in the regenerating and developing pancreas. *J. Endocrinol.* 163, 523–530