The genetic programme of pancreatic β -cells: basic science for the development of β -cell therapy

Workshop on Programming Pancreatic β -Cells

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Introduction

β -cells are located in the pancreatic islets of Langerhans and produce insulin in response to a rise in blood glucose; the destruction or dysfunction of β -cells results in diabetes. Although insulin therapy has proved useful for the treatment of this disease, it is hoped that β -cell-replacement therapy will be even more effective; however, there is a limited supply of donor β -cells. Alternative sources of β -cells for implantation are therefore required and their activation *in situ* needs to be controlled. Possible methods to recreate the development, regeneration and growth of pancreatic β -cells can be learned from naturally occurring processes, such as embryonic β -cell development and adaptation of the β -cell mass in adult life to obesity or other stress conditions. During this workshop, scientists from diverse research fields were brought together to foster discussions on the basic mechanisms of pancreatic development, pancreatic regeneration and growth, transdifferentiation and programmed embryonic stem (ES)-cell differentiation.

Transcriptional regulators of pancreatic development

In recent years research has amassed an impressive set of data describing the mechanisms that control endoderm development, the subsequent morphogenesis of the pancreas and the differentiation of pancreatic cells (Murtaugh & Melton, 2003; Collombat *et al*, 2006; Jensen, 2004). Concurrently, research has also progressed on the transcriptional regulation involved.

The number of known transcription factors involved in β -cell development continues to increase. M. Sander (Irvine, CA, USA) has investigated the expression of SRY-box-containing (Sox) protein family members during pancreatic development, and found that Sox9 is expressed in a subset of progenitor cells. Conditional inactivation of Sox9 in these cells leads to severe pancreatic hypoplasia. G. Gradwohl (Strasbourg, France) and R. Gasa (Barcelona, Spain) have studied the role of neurogenin 3 (Ngn3; Fig 1), which is essential for endocrine progenitor development. They identified several transcriptional regulators that are targets of Ngn3, including the transcription factor atonal homologue 8 (also called Math6). The musculoaponeurotic fibrosarcoma (Maf) oncogene family of

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transcription factors is another emerging group that is relevant to β -cell development. R. Stein (Nashville, TN, USA) showed that MafB is expressed transiently in developing β -cells and permanently in α -cells, and is necessary for insulin and glucagon expression. In

◀ **Fig 1** | Differentiation of pancreatic β -cells during development, in adult transdifferentiation and regenerative processes, and *in vitro*. Question marks indicate conflicts reported during the meeting or in previous publications. The phenotypic similarities and differences between actual β -cells and those generated *in vitro* need to be investigated further. The genes depicted in red and green are markers of the different stages. The meanings of the letters in brackets are explained in the main text. The immunofluorescence image of pancreatic buds was kindly provided by P. Serup and M. C. Jørgensen. Cxcr4, chemokine (C-X-C motif) receptor 4; ES cell, embryonic stem cell; Ngn3, neurogenin 3; Nkx6.1, NK6 transcription factor-related locus 1; Pdx1, pancreatic and duodenal homeobox factor 1; Sox, SRY-box-containing protein.

the *MafB*-knockout mouse, expression of other transcription factors remained unaffected in the α -cells and β -cells. Conversely, MafA is β -cell-specific and genetically complements MafB in the process of islet organization. A. Ait-Lounis of the W. Reith laboratory (Geneva, Switzerland) reported that mice deficient in the transcription factor regulatory factor X, 3 (Rfx3) exhibit an expansion of the pancreatic polypeptide-expressing cells at the expense of insulin-expressing cells, implying that this factor is a regulator of endocrine cell fate.

Various parameters, such as the amount, timing and duration of transcription-factor activity, have been investigated. R. MacDonald (Dallas, TX, USA) showed that sustained pancreatic and duodenal homeobox factor 1 (Pdx1; Fig 1) activity is required for the emergence of endocrine cells. C. Wright (Nashville, TN, USA) manipulated the *Pdx1* promoter to engineer mice expressing graded levels of Pdx1, and showed that specific levels of Pdx1 activity are required for the development of the pyloric sphincter, the dorsal and ventral pancreas, and β -cells. These experiments pave the way for the study of transcription factors with multiple functions in the pancreas. Ngn3 is one such factor, as illustrated by A. Grapin-Botton (Lausanne, Switzerland) who expressed a tamoxifen-inducible Ngn3 variant in *Ngn3*-null mice to show that rescue of Ngn3 activity at different time points induces different endocrine cells. In addition, she showed that the transcription factor islet 1 (Isl1), which is required at the onset of pancreatic and endocrine development, also controls later stages of endocrine development. Indeed, C. L. May (Philadelphia, PA, USA) showed that mice with a Pdx1-Cre-mediated conditional knockout of Isl1 are glucose intolerant, which suggests a cell-autonomous function of Isl1 in endocrine cells.

The elucidation of the transcriptional network that leads to β -cell differentiation is still in its infancy. By contrast, the network that operates during liver development has been characterized to a greater extent and contains many factors that function in the pancreas. Studies on liver development were therefore also presented at the meeting. L. Lokmane of the S. Cereghini laboratory (Paris, France) showed that the transcription factor variant hepatic nuclear factor 1 β /transcription factor 2 (vHNF1 β /Tcf2) is necessary not only for the formation of the dorsal pancreatic bud, but also for liver-bud specification and expansion. The group of I. Talianidis (Heraklion, Greece) performed an impressive set of chromatin-immunoprecipitation experiments to determine how transcription factors regulate each other. The results showed that they constitute autoregulatory and cross-regulatory circuits, the complexity and stability of which increase during development. In addition, using chromatin immunoprecipitation combined with microarray analysis of the immunoprecipitated fragments, D. Odom (Cambridge, MA, USA) compared the

binding sites of the transcription factors HNF1 α , HNF4 α and HNF6 in the human and mouse genome, and showed that transcriptional regulation displays an unexpectedly small amount of interspecies conservation. However, the extent of binding-site conservation is large within genes belonging to specific Gene Ontology categories.

Transcription-factor binding was also investigated at the subnuclear level by R. Luco of the J. Ferrer laboratory (Barcelona, Spain). By using an elegant combination of immunostaining with fluorescent *in situ* hybridization, she studied HNF1 α and showed that it determines the positioning of target loci to subnuclear compartments enriched in active chromatin modifications. J. M. Servitja (Barcelona, Spain), from the same laboratory, reported that in pancreatic islets, HNF1 α depletion affects the expression of only some of its direct target genes, indicating that promoter occupancy does not necessarily correlate with functional requirement. However, this requirement is tissue-specific, so the transcription of a gene bound by HNF1 α in two tissues might be dependent on this factor in only one of the tissues.

During pancreatic development, the ducts give rise to exocrine acini and the endocrine cells delaminate from the ductal epithelium. Therefore, there has been research into the transcriptional control of duct development. S. Leach (Baltimore, MD, USA) showed that the decision to differentiate into exocrine or endocrine cells requires the Notch signalling pathway acting on the expression of Ngn3 and the pancreas-specific transcription factor 1 (Ptf1). F. Lemaigre (Brussels, Belgium) studied HNF6-knockout mice and showed that this transcription factor controls endocrine-progenitor development as well as morphogenesis of the pancreatic ducts. A. Merlos-Suárez of the P. Real laboratory (Barcelona, Spain) has analysed the transcriptome of human duct cells. This more global approach is expected to define a ductal-cell signature that will allow the issues related to the development of endocrine islets from the ducts to be addressed. In addition, M. Hebrok (San Francisco, CA, USA) presented elegant live-imaging data from cultured pancreatic explants expressing green fluorescent protein, and showed how branching of ducts occurs and β -cells coalesce to form islets. This data set, and the new tools that have been developed, are likely to initiate new research on islet morphogenesis.

Signalling and pancreatic development

Pancreatic and β -cell development also rely on the reception of extracellular signals. Recent attention has largely focused on the fibroblast growth factor (FGF) and wingless-related-MMTV integration site (Wnt) pathways. K. Zaret (Philadelphia, PA, USA) has previously shown that FGF2 signalling from the cardiac mesoderm must be repressed for the specification of the ventral pancreas. When FGF2 is maintained, the liver anlage develops at the expense of the ventral pancreas. Zaret has now deciphered the intracellular pathways that mediate signalling and showed that hepatic induction relies on activation of the mitogen-activating protein kinase rather than the phosphoinositide 3-kinase pathway. J. Wells (Cincinnati, OH, USA) further highlighted the complexity of FGF signalling during pancreatic induction. He showed that, in addition to signalling to the liver and ventral pancreas, an FGF signal—possibly FGF2—might be sent by the notochord to the dorsal pancreas. Furthermore, graded FGF signalling—possibly from FGF4—positions the organs along the antero-posterior axis of the digestive tract. This signalling is positively autoregulated through the expression of the FGF-binding protein 1, which enhances FGF ligand solubility. The zebrafish was used as an experimental model by members of the

group of B. Peers (Liège, Belgium), who reported knockdown experiments indicating that FGF24—a member of the FGF8 family—might be important for the specification of the anterior–ventral pancreatic bud. Finally, R. Scharfmann (Paris, France) found that FGF10 is secreted by the mesenchyme, and triggers the expansion of multipotent pancreatic progenitors expressing Pdx1.

The role of the Wnt pathway in pancreatic development might be similarly complex. Through its pancreas-specific inactivation, β -catenin was recently shown to be necessary for the maintenance of pancreatic progenitors, although there is some controversy as to whether this is due to its activity in the Wnt pathway or in cell adhesion. Hebrok used constitutively active β -catenin, and A. Strom of the P. Herrera laboratory (Geneva, Switzerland) specifically inactivated the anaphase-promoting complex (APC) in the pancreas, to show that the Wnt signalling pathway must be inactive or at a low level of activity for appropriate exocrine pancreatic expansion after birth. The accumulation of β -catenin in APC-knockout pancreatic cells does not affect antenatal development, but leads to a fourfold expansion of the exocrine pancreas in the first postnatal month, which is a process that ceases in older animals. The mechanisms by which the embryonic pancreas and endocrine cells are resistant to APC loss are still unclear. The pathway might be kept at a low activity in the pancreas of older animals by the induction of negative regulators of β -catenin signalling (Strom). In the islets, β -catenin shuttling from the cytoplasm to the nucleus seems to be tightly controlled and, in ageing animals, nuclear translocation leads to the loss of insulin in islets (Hebrok). The opposing phenotypes of β -catenin activation at early and late stages of pancreatic formation reported by Hebrok imply that the Wnt pathway might also be active earlier in pancreatic development than previously thought. This was shown more directly in zebrafish, in which Wnt2 seems to control the merger of the ventral–anterior and dorsal–posterior (endocrine) pancreatic buds (J. Rhee & S. Leach, Baltimore, MD, USA).

Adult β -cell regeneration

In adults, β -cells can be generated by stem-cell differentiation, proliferation of pre-existing or newly formed β -cells, and trans-differentiation from other cell types (Bonner-Weir & Weir, 2005). During this workshop, *in vivo* models involving the genetic or chemical ablation of β -cells were presented to study their regenerative ability and the origin of newly formed β -cells. For example, expression of diphtheria toxin A (DTA)—an inhibitor of eukaryotic protein synthesis—under the control of the insulin promoter in mice (β -DTA), allows the effects of efficient and cell autonomous β -cell ablation to be studied without inflammation. As reported by Y. Dor (Jerusalem, Israel), β -DTA mice recover from hyperglycaemia within 10–20 weeks after interruption of toxin expression, and lineage tracing of the surviving β -cells revealed that the regeneration is largely based on proliferation (Fig 1, arrow a). By sequential application of 5-chloro-2'-deoxyuridine and 5-iodo-2'-deoxyuridine to the drinking water of mice, J. Kushner (Philadelphia, PA, USA) differentially labelled the DNA of dividing cells and showed that under normal physiological conditions a specific pool of highly replicative β -cells does not exist, but rather all β -cells exhibit an equal proliferation potential. This technology is expected to be useful for studying the kinetics of β -cell cycling under conditions of regeneration.

Several studies have focused on β -cell proliferation, including work from A. Bhushan (Los Angeles, CA, USA), who reported that Skp2-mediated regulation of p27 turnover by the Skp1/cullin/

F-box (SCF) ubiquitin ligase controls β -cell proliferation in the normal adult mouse pancreas. The activity of this regulatory pathway in β -cell division during regeneration remains to be investigated.

A different mouse model for β -cell regeneration following chemical ablation by the β -cell toxin alloxan was developed by H. Heimberg (Brussels, Belgium). Both inflammatory and angiogenic signals support a rapid regeneration of the β -cell mass by stem/progenitor differentiation, rather than by proliferation of the surviving β -cells. The—still preliminary—information from the different models emphasizes the plethora of pathways that cope with an insufficient β -cell mass.

The mechanisms of β -cell proliferation have been further explored *in vitro*. S. Efrat (Tel Aviv, Israel) showed that when adult human islet cells were expanded 65,000-fold in culture, they lost most of their β -cell-specific proteins, including insulin. The expanded cell population could be redifferentiated by the addition of betacellulin—a member of the epidermal growth factor (EGF) family—to the medium (Fig 1, arrow b). However, the success of this method is still unpredictable and strongly donor-dependent.

Besides islet (progenitor) cells, several other types—both inside and outside the pancreas—have been tested as candidate β -cell precursors. D. Stoffers (Philadelphia, PA, USA) used lineage-tracing experiments to show that the acinar cell compartment is not a source of β -cells during the *in vivo* regeneration of an injured pancreas (Fig 1, arrow c). However, L. Bouwens (Brussels, Belgium) showed that when rat acinar cells were cultured *in vitro* together with the EGF and leukaemia-inhibitory factor (LIF), they initially dedifferentiated then redifferentiated into functional β -cells (Fig 1, arrow d). Research by J. Slack (Bath, UK) on reprogramming liver cells to become β -cells (Fig 1, arrow e) coincidentally revealed that the biliary epithelium is an ectopic site for the formation of endocrine islet cells, which might provide another source of cells able to transdifferentiate into β -cells (Fig 1, arrow f).

All of these experimental models will probably become important tools for investigating the nature of the regenerative signals that determine the β -cell mass. Updates on the main extracellular signals that determine adaptation of the β -cell mass in the adult pancreas were presented at the meeting. The effects of the complex signalling pathways involving insulin, insulin-like growth factor 1/2 (IGF1/2) and glucagon-like peptide 1 (GLIP1) are being carefully dissected. For example, B. Thorens (Lausanne, Switzerland) showed that preventing the downregulation of GLIP1-regulated genes by knockdown of its inhibitors stimulated the glucose-induced proliferation of β -cell lines. However, as these growth factors affect a broad spectrum of cell types, specific targeting of the β -cell is a major challenge. M. White (Boston, MA, USA) further focused on signalling in β -cells. Insulin, IGF and GLIP1 all use the insulin receptor-substrate 2 (IRS2) to signal in β -cells. White reported the delayed appearance of diabetes in non-obese diabetic (NOD) mice with increased IRS2 expression in β -cells. These results are an incentive to search for β -cell-specific drugs that control this signalling pathway.

Differentiation of ES cells

A potential source of β -cells for the treatment of diabetes is the programmed differentiation of ES cells. Ideally, this should recapitulate natural processes and some progress has been made towards achieving this goal (D'Amour *et al*, 2006), despite the fact that mature and functional β -cells have not yet been produced. More knowledge of developmental biology is required to uncover the mechanisms that underlie β -cell differentiation

P. Serup (Gentofte, Denmark) summarized the principles of how cells from the inner-cell mass—from which ES cells derive—progressively give rise to the primitive streak and definitive endoderm (Fig 1, arrow g). By using a battery of reporter lines, he showed how combinations of growth factors of the activin, Wnt and bone-morphogenic protein (BMP) families, used at the appropriate concentration when added to mouse ES-cell cultures, allow the recapitulation of the induction of marker genes for the primitive streak and definitive endoderm. Similarly, H. Semb (Lund, Sweden), who works with human ES lines, showed how markers of the mesendoderm and definitive endoderm could be induced, and proposed that the best criterion for the identification of pancreatic precursors is the co-expression of NK6 transcription factor-related locus 1 (Nkx6.1) and Pdx1 (Fig 1, arrows g and h). M. Medina of the J. Diez laboratory (Miami, FL, USA) described a similar approach, combined with the purification of definitive endoderm cells with anti-chemokine (C-X-C motif) receptor 4 (CXCR4) antibodies (Fig 1, arrow g). Research on ES-cell differentiation has also benefited from information on antero-posterior patterning of the endoderm in the embryo, which allows the appropriate selection of growth factors at optimal concentrations (Fig 1, arrow h). The importance of endoderm patterning was also underscored by J. Wells (Cincinnati, OH, USA), who mentioned that the protocol designed by D'Amour *et al* (2005) to differentiate ES cells into endoderm might preferentially give rise to anterior endoderm (Fig 1, arrow g).

It is possible to differentiate ES cells by using combinations of growth factors; other approaches include the forced expression of transcription factors. J. Odorico (Madison, WI, USA) described ES-cell lines with tetracyclin-dependent inducible expression of pancreatic transcription factors and showed how they can contribute to the understanding of the pancreatic network of transcription factors. A combined approach was followed by A. Bernardo (of the K. Docherty laboratory, Aberdeen, UK), in which ES cells expressing a tamoxifen-inducible variant of Pdx1 were cultured with growth factors and induced to express pancreatic markers. Finally, a similar approach was reported by A. Skoudy (Barcelona, Spain), who combined forced expression of transcription factors in mouse ES cells with their culture in conditioned media from fetal pancreatic rudiments, which allowed the generation of acinar-like cells.

Conclusions

Although considerable progress has been made in understanding the mechanisms that regulate β -cell development, proliferation and regeneration, as well the programmed differentiation of β -cells from various sources, several issues remain unsolved. These include the configuration of the epigenetic code in the β -cells, the stability of their phenotype, the morphogenesis of the islets, the reproducibility of the differentiation processes and their resemblance to fetal rather than mature β -cells. It will also be difficult to decide on the most efficient way to channel the available information into pre-clinical models. We look forward to hearing more about these issues at future meetings.

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REFERENCES

- Bonner-Weir S, Weir GC (2005) New sources of pancreatic β -cells. *Nat Biotechnol* **23**: 857–861
- Collombat P, Hecksher-Sorensen J, Serup P, Mansouri A (2006) Specifying pancreatic endocrine cell fates. *Mech Dev* **123**: 501–512
- D'Amour KA, Agulnick AD, Eliazar S, Kelly OG, Kroon E, Baetge EE (2005) Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat Biotechnol* **23**: 1534–1541
- D'Amour KA, Bang AG, Eliazar S, Kelly OG, Agulnick AD, Smart NG, Moorman MA, Kroon E, Carpenter MK, Baetge EE (2006) Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol* **24**: 1392–1401
- Jensen J (2004) Gene regulatory factors in pancreatic development. *Dev Dyn* **229**: 176–200
- Murtaugh LC, Melton DA (2003) Genes, signals, and lineages in pancreas development. *Annu Rev Cell Dev Biol* **19**: 71–89

From left to right: Anne Grapin-Botton, Harry Heimberg & Frédéric Lemaigre