Insulin gene regulation and islet development as studied in genetically modified tumors and transgenic laboratory animals: A review.

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Introduction

Insulin is essential for maintenance of blood glucose homeostasis. The insulin gene is only transcribed in the β-cell of the pancreatic islet of Langerhans. Insulin dependent diabetes mellitus (IDDM) is characterized by an autoimmune destruction of the β-cells and IDDM-patients thus require a lifelong treatment with exogenous insulin (Figure 1). In addition to the insulin producing β-cell the islet of Langerhans is composed of three other highly specialized cell types which each produce a particular hormone. The four cell types are presumed to be derived from common precursor cells but only the β-cells are destroyed in IDDM (Figure 1). The β-cell is an uniquely specialized insulin factory which can sense blood glucose and secrete appropriate amounts of insulin in response to changes in glucose levels (for review see (Orci, Vassalli, & Perrelet, 1988)). Part of this specialization includes the ability to transcribe the insulin gene to an extend which is

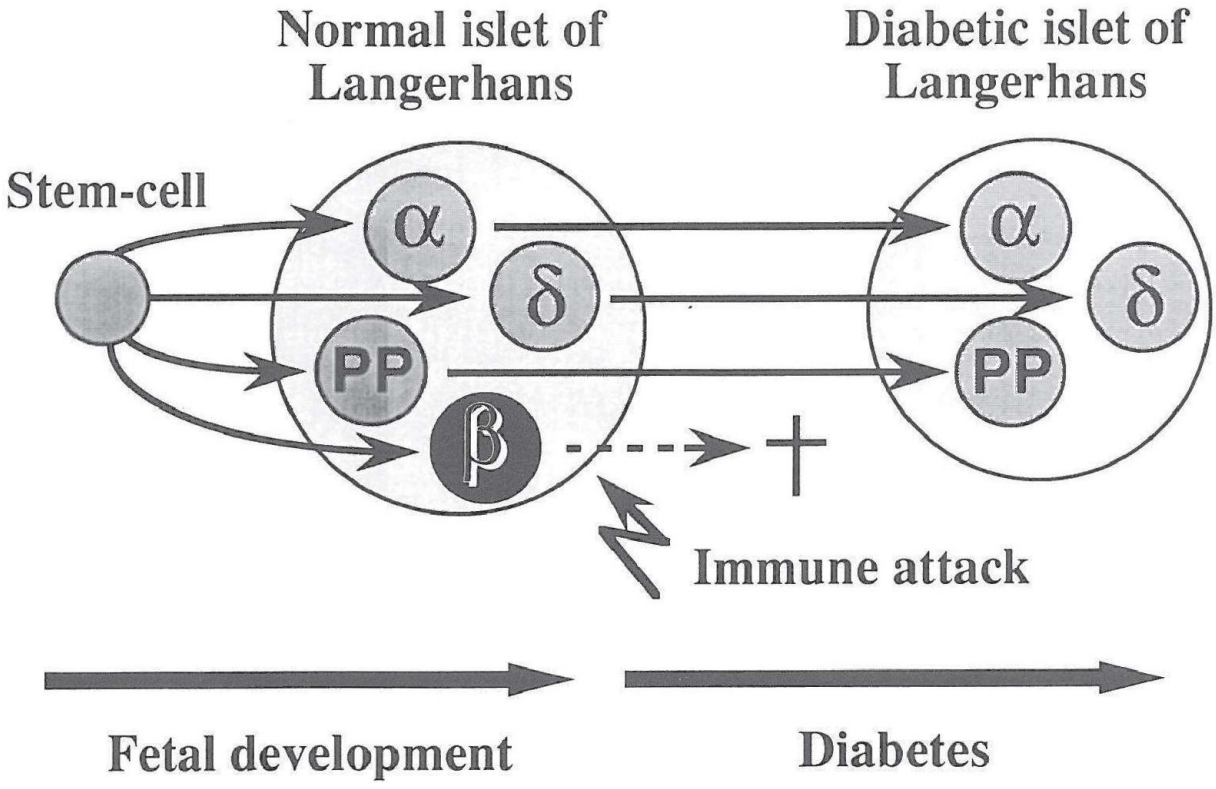

Figure 1. Islet development in relation to autoimmune type I diabetes. The four islet cell phenotypes are thought to develop from common precursor cells. Only the insulin producing β-cell is destroyed in autoimmune type I diabetes (IDDM), while the α- (glucagon) δ- (somatostatin) and PP-cell (pancreatic polypeptide) are left intact.

hardly matched by other genes of specialized cells. Moreover, the rate of insulin gene transcription is directly regulated by glucose (Brunstedt & Chan, 1982; Efrat, Surana, & Fleischer, 1991).

The molecular mechanism responsible for the tissue specific insulin gene regulation has been studied extensively over the past few years. Such knowledge is an important piece of the puzzle of understanding islet cell differentiation in general.

The use of transgenic animals, as well as transplantation of pluripotent islet tissue have been important tools in such studies and is reviewed in this paper in relation to pancreatic islet development and insulin gene regulation (left part of Figure 1). A number of transgenic approaches have also been taken to study the possible mechanisms of β-cell destruction and development of IDDM (right part of Figure 1) and include targeted β-cell expression of various immune modulating molecules such as MHC classII (Lo, Burkly, Widera, Cowing, Flavell, Palmiter, et al., 1988) and the cytokines interferon-γ (Sarvetnick, Liggitt, Pitts, Hansen, & Stewart, 1988) TNF-a (Higuchi, Herrera, Muniesa, Huarte, Belin, Ohashi, et al., 1992) and interferon -α (Stewart, Hultgren, Huang, Pitts-Meek, Hully, & MacLachlan, 1993). These aspects will not be covered further.

The insulin gene promoter is a powerful tool in the targeting of gene products to the β-cell
Early in vitro studies by Rutter and co-workers (Edlund, Walker, Barr, & Rutter, 1985; Walker, Edlund, Boulet, & Rutter, 1983) showed that the immediate 5' flanking sequence of the rat I insulin gene was sufficient and responsible for selective expression of a reporter gene in transfected insulinoma cells. Moreover, genomic sequencing of the two non-allelic insulin genes (ins I and ins II) in rats and mice revealed that ins I most likely originated from an aberrant transcript of the ins II where only one of two introns was excised but contained approx. 500 bases upstream of the usual start site. Such a transcript was believed to be translated to cDNA before integration, – an event which took place before mice and rats segregated as independent species more than 25 million years ago (Soares, Schon, Henderson, Karathanasis, Cate, Zeitlin, et al., 1985). Since both genes are transcribed with similar efficiency and with similar tissue restriction it follows that these 500 bases of the 5'-flanking region most likely contain all information necessary for this regulated expression.

This assumption was elegantly proven by D. Hanahan in 1985 where he used 650 base pairs of the rat II insulin gene promoter/enhancer in front of the coding sequence for the viral oncogene, SV40-large T antigen, and produced transgenic mice which targeted expression of T-antigen to the pancreatic β-cell (Hanahan, 1985). Moreover, these mice produced heritable β-cell tumors from which very important in vitro cultures (β-Tc) have been derived (Efrat, Linde, Kofod, Spector, Delannoy, Grant, et al., 1988; Efrat, et al., 1991). The same transgenic construct was also used to generate β-cell lines from the NOD mouse which suffers from spontaneous Type I diabetes (Hamaguchi, Gaskins, & Leiter, 1991). Similarly, the 850 bp 5'-flanking glucagon promoter/enhancer sequence was utilized in transgenic mice to transform glucagon producing α-cells from which in vitro cultures (α-Tc) were derived (Efrat, Teitelman, Anwar, Ruggiero, & Hanahan, 1988).

Transient phenotypes and co-expression of hormones during pancreas ontogeny.
A detailed study on T-antigen expression in the above transgenic mice with heritable β-cell tumors revealed that insulin and T-antigen gene expression was not necessarily coordinated during the early fetal pancreatic development although presumed to be controlled by the same gene regulatory element. The outcome of these studies was the propo-

sal of a cell-lineage model (The Teitelman model, Figure 2) based on the discovery that early pancreas ontogeny was characterized by multihormonal endocrine phenotypes (Alpert, Hanahan, & Teitelman, 1988). Glucagon is the first hormone appearing as detected by immunocytochemistry. It is followed by insulin, somatostatin and pancreatic polypeptide in sequential order. More recent transgenic studies have shown that glucagon expression is actually preceded by peptide YY expression (Upchurch, Aponte, & Leiter, 1994). During this period of development the hormones are coexpressed in various combinations while in the mature and fully differentiated phenotypes of the adult islet only one of the classical islet hormones are expressed in a given cell. Similar multihormonal phenotypes have also recently been described during rat (Hashimoto, Kawano, Daikoku, Shima, Taniguchi, & Baba, 1988) and human islet development (DeKrijger, Aanstoot, Kranenburg, Reinhard, Visser, & Bruining, 1992; Larsson & Hougaard, 1994).

Studies by us and others revealed that endocrine cell lines although of clonal origin were often heterogeneous and produced multiple hormones (Madsen, Larsson, Rehfeld, Schwartz, Lernmark, Labrecque, et al., 1986; Philippe, Chick, & Habener, 1987). We thus proposed that MSL-cells which were derived from a liver metastasis of an X-ray induced transplantable islet tumor represented transformed islet stem cells (Chick, Warren, Chute, Like, Lauris, & Kitchen, 1977; Madsen, et al., 1986). In subsequent studies this was sub-

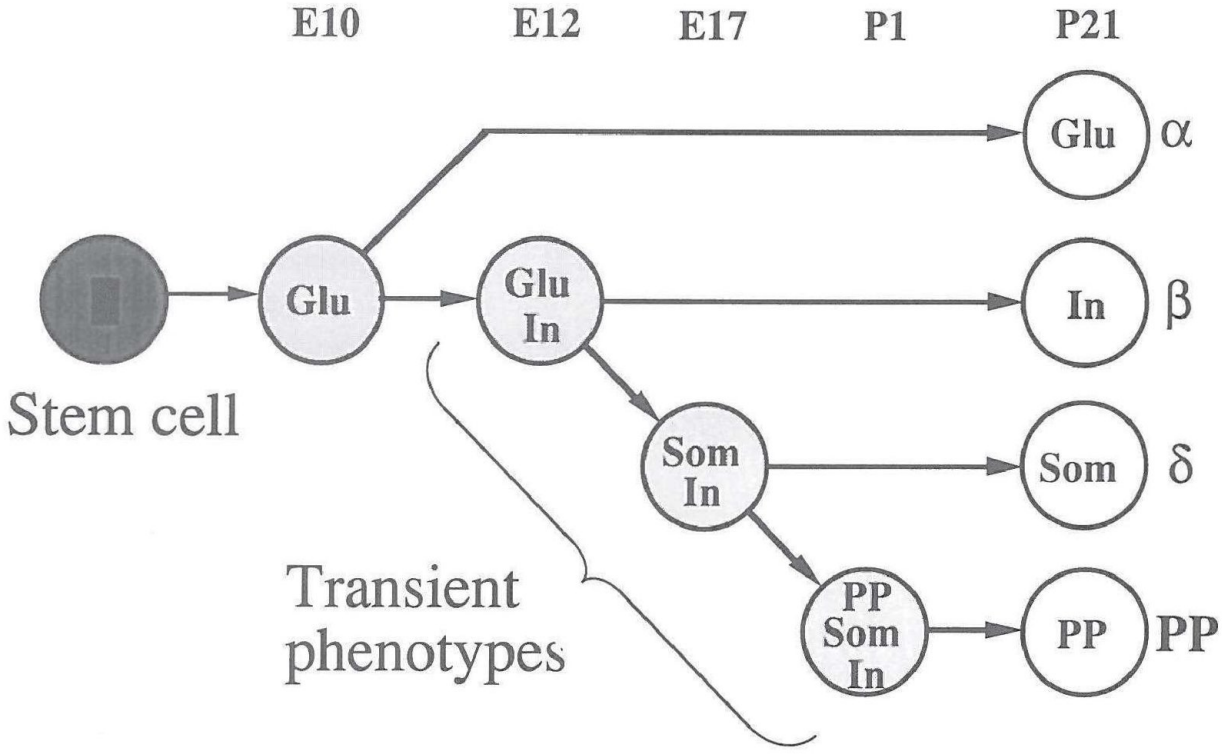

Figure 2. The Teitelman model of mouse islet development depicting a cell lineage relationship between the different mature phenotypes. Figures above (E10, 12, and 17) denote days of gestation while P1 and P21 represents days after birth. As seen glucagon appears first as detected by immunocytochemistry, which is then followed by insulin, somatostatin and pancreatic polypeptide in sequential order [adapted from (Alpert, et al., 1988)].

stantiated by the derivation of stable transplantable insulinomas, glucagonomas and an in vitro somatostationoma of common clonal origin (Madsen, Andersen, Michelsen, Owerbach, Larsson, Lernmark, et al., 1988; Madsen, Karlsen, Nielsen, Lund, Kofod, Welinder, et al., 1993; Serup, Andersen, Petersen, & Madsen, 1992).

In support of the Teitelman model also other endocrine cells of the intestinal epithelium were shown to pass through a phase characterized by co-expression of several hormones. These studies were carried out using chimeric mice which allowed detailed analyses of ontogeny of the intestinal epithelium. It was shown that the basal crypt-cell was the progenitor for all cells on both sides of the villi ascending from the crypt [for review see (Gordon, 1989)]. It was thus concluded that endocrine as well as the mucus producing epithelial cells were derived from the same stem cell and that the endocrine cells when closer to the crypt (younger cells) co-expressed combinations of hormones (Roth, Kim, & Gordon, 1992).

The islet of Langerhans consists of few thousands of cells and in man the entire pancreas contains roughly one million islets scattered throughout the exocrine parenchyma. Jami and co-workers (Deltour, Leduque, Paldi, Ripoche, Dubois, & Jami, 1991) showed by the use of chimeric animals that a single islet was formed from more than one precursor cell since a high frequency of islets were found to be chimeric with respect the transgene in question (human insulin, see below).

Functional conservation of the insulin enhancer/promoter among mammalian species

Sequence comparisons of 5'-flanking enhancer regions of mammalian insulin genes revealed considerable homology although not as extensive as parts of the exons encoding the conserved insulin a- and b-chains (Steiner, Chan, Welsh, & Kwok, 1985). Transgenic studies showed that these regions were functionally conserved among mouse/rat and man. Thus, when micro-injecting large genomic fragments containing the entire human insulin gene locus then the forming transgenic mice expressed human proinsulin selectively in the pancreatic β-cells (Bucchini, Ripoche, Stinnakre, Desbois, Lores, Monthioux, et al., 1986; Selden, Skoskiewicz, Howie, Russel, & Goodman, 1986). Detailed deletion studies in transgenic mice on the characterization of the human insulin promoter more or less confirmed previous in vitro data (Bucchini, Madsen, Desbois, Pictet, & Jami, 1989; Fromont-Racine, Bucchini, Madsen, Desbois, Linde, Nielsen, et al., 1990).

Similarly, transfected pluripotent MSL cells carrying a 15 kb genomic fragment containing the human insulin gene were able to coactivate this in parallel with the endogenous rat ins I and II genes when induced to form β-cell tumors in vivo (Madsen, et al., 1988). In conclusion, the transacting factors involved in regulating β-cell specific gene expresson apparently recognize particular cis-elements in the insulin promoter/enhancer region from rat, mouse, or human with similar efficacy.

A key regulatory enhancer binding protein: STF1/IPF1/IDX1

Several cis elements have been identified by their ability to interact with nuclear extracts from insulin producing tissue [for review see (Ohlsson, Karlsson, Norberg, Thor, & Edlund, 1989; Philippe, 1991; Philippe, 1994)]. Particular elements with the core sequence TAAT are found in all promoter/enhancer regions of the rat insI and II genes as well as in the human insulin gene (figure 3). These elements are known to be binding sites for homeodomain containing proteins (Kornberg, 1993). A nuclear binding activity termed IPF1 was identified as the major candidate protein present in β-cells (Ohlsson, Thor, & Edlund, 1991). This factor was cloned from a mouse β-Tc cDNA library and con-

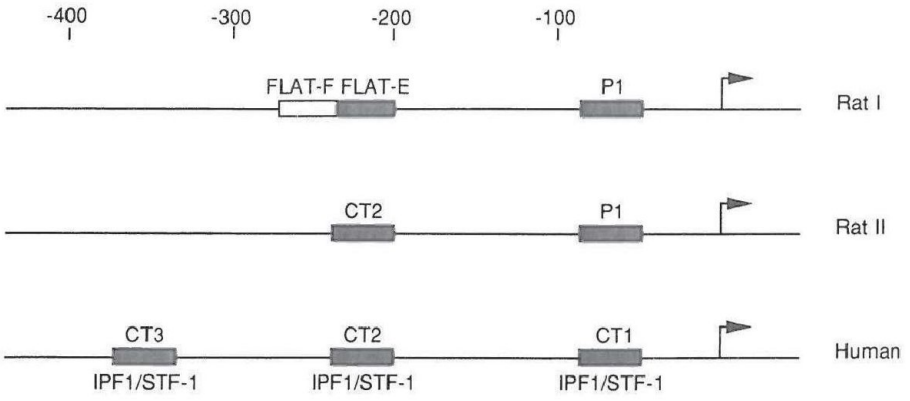

Figure 3. The 5' flanking promoter/enhancer sequences of the human, rat I and rat II insulin genes all contain consensus binding sites (TAAT) for homeobox transcription factors. IPF1/STF1 is the most prominent binding activity present in islet β-cells (Petersen, et al., 1994). Several other cis-elements are present in these promoters and a unifying nomenclature concerning all these elements have been proposed (German, Ashcroft, Docherty, Edlund, Edlund, Goodison, et al., 1995).

firmed to be a homeodomain protein which was not expressed in the glucagon producing α-TC (Ohlsson, Karlsson, & Edlund, 1993). Two other groups independently cloned the homologous rat protein from somatostatin producing cell lines and found it able to also functionally interact with TAAT motifs in the somatostatin promoter (Leonard, Peers, Johnsson, Ferreri, Lee, & Montminy, 1993; Miller, McGehee, & Habener, 1994). Thus STF1 (somatostatin transacting factor 1) (Leonard, et al., 1993) and IDX1 (islet duodenal homeobox factor 1) (Miller, et al., 1994) represents the equivalent rat protein. Since STF1 was cloned from our MSL derived somatostatinoma line which still express a certain amount of insulin we proposed that STF1/IPF1/IDX1 was involved in the transient multihormonal phenotype during development (Petersen, Serup, Leonard, Michelsen, & Madsen, 1994). However, later in development this factor seems to be restricted to the mature β-cell and we have shown that it may be a candidate factor involved in the glucose regulated transcription of the insulin gene, a property of the fully mature β-cell (Petersen, et al., 1994). Docherty and coworkers have recently shown that this factor becomes phosphorylated in response to high glucose levels (MacFarlane, Read, Gilligan, Bujalska, & Docherty, 1994).

Transgenic mice with the IPF1 gene disrupted by homologous recombination was recently generated by H. Edlund and co-workers (Jonsson, Carlsson, Edlund, & Edlund, 1994). Interestingly, these mice lacked the ability to form a pancreas but were otherwise normal. The pups died within few days. None of the two pancreatic anlage were formed while the liver and the bile duct were normal. It may thus be concluded that the IPF1 factor is having several crucial functions during pancreatic development and islet differentiation. It is a fundamental requirement for early pancreas formation and probably a key regulator of the insulin gene in the adult β-cell.

Concluding remarks

The development of transgenic technology have revolutionized the fields of developmental biology. The conventional transgenic technique is a powerful tool in characterizing gene regulatory regions (promoter/enhancer structures) and the insulin gene promoter is an interesting example of a very short se-

quence responsible for a highly tissue-restricted expression. Chimeric mice (mixing transgenic and normal embryonic cells) are highly informative in studies of embryogenesis and organ development and this technique was fundamental in the creation of null mutants by homologous recombination of ES cells. The knock-out of the homeodomain-protein, IPF-1, resulting in a phenotype without pancreas formation (Jonsson, et al., 1994) is opening up new avenues for understanding of the early processes involved in organ development. The functional importance of IPF1 in the pancreatic β-cell later in development can now be approached by the refined technique of tissue specific knock-out experiments based on the Cre-loxP recombination system (Gu, Marth, Orban, Mossmann, & Rajewsky, 1994). In short, via ES cells transgenic mice are generated with loxP sites introduced by homologous recombination into the target gene of interest. The loxP sites are inserted into intron sequences leaving the target gene functional. These mice are then bred with conventional transgenic mice generated by microinjection of Cre-expression vector under the control of a tissue-specific promoter. An insulin promoter-Cre gene would thus be highly useful in mediating β-cell specific knock-out of various target genes furnished with integral loxP sites. Such studies will allow important functional characterization of various transacting factors involved in tissue specific gene regulation and cellular differentiation.

Summary
The pancreatic islet of Langerhans is composed of four highly distinct cell types specialized to mass produce a particular hormone. Insulin is thus the main product released from the islet β-cell in response to elevated glucose. The four cell types maturate during fetal development. Pluripotent rat islet tumors can to a certain degree undergo similar maturation processes when passaged in vivo. Such a model has been used to study the β-cell specific process of insulin gene activation. Transgenic mice have been instrumental in defining the functional regulatory elements involved in restricting the insulin gene activity to the pancreatic β-cell. The tissue-specific enhancer/promoter has thus been identified and used in combination with a series of other genes which in transgenic mice targets expression of the gene in question selectively to the β-cell. Important transacting factors have been identified and cloned which are in part responsible for mediating tissue specific insulin gene expression. One such factor when »knocked-out« results in a phenotype lacking the entire pancreas. Future developments in targeting »knock-out« of genes to particular cell types will help dissecting out the multiple functions of such regulatory transacting factors.

Sammendrag
Den Langerhanske i pankreas består hovedsagelig af fire forskellige celletyper, som hver især er specialiserede til at masseproducere og sekretere et bestemt hormon. Insulin er således hovedproduktet, som frisættes fra β-cellen ved stigende blodsukkerniveau. De fire celletyper modnes under den føtale udvikling. Pluripotente rotte -celle tumorer kan til en vis grad eftergøre dette udviklingsmønster når de passeres in vivo. En sådan model har været anvendt til at studere den β-cellespecifikke proces, hvorunder insulin genet aktiveres. Brugen af transgene mus har været overordentlig vigtig i karakteriseringen af de funktionelle regulatoriske elementer, som er ansvarlig for den vævsspecifikke insulinekspression i β-cellen. Denne promoter/enhancer struktur er således kendt og har været anvendt i et væld af kombinationer med andre gener, som således udtrykkes selektivt i β-cellen i transgene mus. Vigtige transaktiverende faktorer, som indgår i reguleringen af den vævsspecifikke insulingen ekspression, er i dag kendte. En af de mest bemærkelsesværdige faktorer har vist sig ved homolog rekombination »knock-out« også at være ansvarlig for pankreas dannelse i det hele taget. Fremtidige udviklinger i organ-specifik »knock-out« vil kunne anvendes til at uddissekere sådanne multi-funktionelle regulatoriske proteiners effekter.

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