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CELL CYCLE CONTROL OF PANCREATIC BETA CELL PROLIFERATION

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1. ABSTRACT

Diabetes mellitus ensues as a consequence of the body's inability to respond normally to high blood glucose levels. The onset of diabetes is due to several pathological changes, which are a reflection of either the inability of the pancreatic beta cells to secrete sufficient insulin to combat the hyperglycemia or a state of insulin resistance in target tissues. However, the significance of changes in beta cell mass and decreased beta cell proliferation or growth in progression of diabetes has been under-appreciated. Beta cells, like all other cells of our body are under the regulatory checks and balances enforced by changes in cell cycle progression. However, very little is known regarding the key components of the cell cycle machinery regulating cell cycle control of beta cells. Knowledge of key elements involved in cell cycle regulation of beta cells will go a long way in improving our understanding of the replication capacity and developmental biology of beta cells. This information is essential for us to design new approaches that can be used to correct beta cell deficiency in diabetes. This review focuses on the current knowledge of factors important for proliferation of beta cells and proposes a cell cycle model for regeneration of the beta cell population lost or reduced in diabetes.

2. INTRODUCTION

Diabetes has long been acknowledged as a hereditary disease on the basis of a relatively high rate of familial transmission. This is corroborated by the observation that the risk of being a diabetic sibling or a child of a person afflicted with the disease is 7% and 6%, respectively (1). The extensively documented polygenic inheritance pattern of diabetes suggests that genetic alterations in two or more predisposition genes could lead to the eventual clinical manifestation of the disease. A fortuitous combination of these predisposition genes, together with environmental risk factors provokes the onset of diabetes. Many of the predisposition genes contribute to the diabetes pathology which includes : beta cell destruction, primarily due to immune destruction; peripheral insulin resistance in target tissues; defective or insufficient insulin secretion in response to stimulators such as glucose; defective pro-insulin synthesis or processing to mature insulin and decreased beta cell proliferation or growth. All of the above outlined pathologic alterations in diabetes have been a focus of expert reviews (2-6). This review will focus on the normal regulation of beta cell mitogenesis and examine factors regulating the proliferation capabilities of beta cells. Capacity of beta

cells to proliferate, like other cell types, reflects the ability of cells to progress normally through the cell cycle. Defects or anomalies in proteins governing the regulated progression through the cell cycle may impair the capacity of beta cells to proliferate under conditions of increased functional demand on the beta cell mass, as is the case during hyperglycemia in diabetes. Beta cell proliferation has been investigated extensively and is also reviewed elsewhere (7-12). However, the role of cell cycle proteins in modulating the proliferative capacity and regeneration potential of beta cells has not been discussed at any level of detail. Modulation of cell cycle pathways in beta cells can provide alternative approaches to repopulate the beta cell population reduced or lost in diabetes patients and will foster development of probable therapies aimed at modifying cell cycle pathways to prevent, reverse or delay the complications of diabetes.

3. DIABETES

3.1. The disease

Diabetes, a disease whose mention goes far back as 1500-3000 B.C and is documented in ancient Greek and Hindu writings, is among the top-ten causes of deaths in Western Nations and the 8th leading cause of death in the United States (1,2). It is a disease that can arrive during the budding years (juvenile diabetes) or later (maturity or late-onset diabetes) in life. In either case, the life threatening complications associated with the disease remain the same. Despite being one of the oldest documented diseases, complete cure for the disease is still elusive which is primarily due to lack of a complete understanding of the disease. Diabetes ensues due to the inability of the body to effectively regulate the sugar balance leading to severe complications such as hyperglycemia (high blood glucose), obesity, neuropathy, nephropathy, retinopathy, limb disorders, bone disorders such as osteoporosis, coma and sometimes untimely death. The beta cells of the pancreas produce a protein, insulin, which monitors glucose levels in the body. Normally, the extra-cellular concentration of glucose is restricted within a very narrow range, irrespective, of variations in glucose availability and utilization. Homeostatic control of normal glucose level is achieved by co-ordinate secretion of insulin and glucagon. The basal rate of glucose utilization is approximately 10 grams per hour and to prevent hypoglycemia due to this utilization of glucose, the liver, the only source of endogenous glucose production, synthesizes glucose at a rate of 10 grams per hour. Approximately 75% of the hepatic glucose production is regulated by levels of glucagon, a product of pancreatic islet-alpha cells. Metabolic demands, such as exercise and fasting, determine the utilization levels of glucose. During exercise, if glucose utilization rises to approximately 50 grams per hour, hepatic production will counter the increased rate of glucose utilization by increasing the rate of glucose production to 50 grams per hour. Also, after a meal the increased glucose uptake is matched by insulin-mediated uptake of the ingested glucose by muscle and fat. At the same time, any further hepatic production of glucose is inhibited by insulin-induced inhibition of glucagon secretion. This insulin response to glucose levels limits hyperglycemia after a meal to a maximum of 10mM and restores it to the 5mM fasting range within two hours. Dysfunction of the alpha and beta cells results in a disordered glucose homeostasis. If the beta cells do not respond to increased levels of glucose, hyperglycemia ensues where glucose levels exceed 10mM, a diagnostic feature of diabetes mellitus. Conversely, beta-cell over-activity, observed in the case of insulinomas or beta cell tumors, leads to hypoglycemia with a possibility of brain cell injury and death.

In diabetic individuals, the regulation of glucose levels by insulin is defective, either due to defective insulin production (Type I diabetes and in some cases of Type II diabetes) or due to insulin resistance (Type II and some cases of Type I). The resultant elevation of blood glucose levels leads to many or all of the complications listed above. In the United States, prevalence of diabetes is approximately 2% of the population of which 10-25% develop diabetes due to an obliteration of their insulin-secreting beta cells by autoimmune destruction. The disorder is referred to as insulin-dependent diabetes mellitus (IDDM), since patients have to rely on insulin injection therapy to prevent hyperglycemia, diabetic ketoacidosis, coma and death due to insulin deficiency (2-4). The remaining 75-90% of diabetic patients suffer from non-insulin dependent diabetes mellitus (NIDDM) which is a result of the inability of the apparently normal beta cells to respond to the hyperglycemia with an increased insulin secretion and insulin resistance in target tissues (2,5,6).

3.2. Type 1 Diabetes or IDDM

Although, diabetes mellitus is defined simply on the basis of the ensuing hyperglycemia, it is a highly heterogeneous disease. The two forms of diabetes, IDDM and NIDDM were distinguished in the late 1960s. This was followed by a realization that IDDM, presumably, had an autoimmune origin (3,4). IDDM is a multifactorial disease with a polygenic inheritance. The genotype of the major histocompatibility complex (MHC) is the strongest genetic determinant. Several aspects of the etiology of IDDM, including the origin and pathogenesis of IDDM, importance of genetic predisposition, interactions of environmental factors and characterization of the anti-beta cell immune response have been reviewed extensively (2-4). Much of the current understanding of IDDM is based on studies using animal models, which serve as excellent tools for genetic and immunological manipulations that are impossible to carry out in human beings.

3.2.1. Spontaneous animal models of IDDM

3.2.1.1. The non-obese diabetic (NOD) mouse model for Type I diabetes

The NOD mouse, a spontaneous model, was discovered in Japan in the late 1970s (13,14). This mouse model has become the prototype for understanding IDDM and was distributed worldwide for research. Diabetes in the NOD mouse usually appears between 4-6 months of age and has a sex-bias with females being more susceptible to developing the disease. The onset of diabetes in this model is a two stage process: overt clinical diabetes by 4-6 months, which is characterized by rampant destruction of

beta cells, is preceded by infiltration of the pancreatic islets with mononuclear cells (insulitis) which occurs at about 1 month of age. In addition to the diabetes, these mice present thyroiditis, sialitis and later in life autoimmune hemolytic anemia. Extensive research on the NOD mouse model forms the basis for understanding the autoimmune nature of IDDM in humans. Recently, new experimental variations of the NOD model have been developed and characterized. These models include, a model for accelerated diabetes induced by cyclophosphamide, an alkylating agent used as an immunosuppressive drug (15). Two injections of 200 mg/kg, one per week in a span of two consecutive weeks, induce diabetes in most male and female mice within 2-3 weeks. This induction of the diabetic phenotype is believed to be through a mechanism involving elimination of regulatory T-cells. The NOD/nude mouse model has also been described where the nude (athymic) genotype has been backcrossed into the NOD genetic background (16). The NOD/SCID mouse has been described where, a mutant gene encoding a defect common to both site-specific DNA recombination and DNA repair pathways was introduced into the NOD genome leading to severe combined immunodeficiency (17). These models, along with the parental NOD mouse model, serve as the basis for many studies exploring the pathogenesis and complications of IDDM.

3.2.1.1. The BB rat

Similar to the NOD mouse model, the BB rat is another spontaneous animal model which has provided clues to the etiology of IDDM (18). The BB rat was initially developed in Canada in the early 1970s. Severe diabetes in the BB rat occurs by 4 months of age and is preceded, as in the NOD mouse, by insulitis. Also, similar to a few diabetic NOD mice sub-strains, the BB rat is accompanied by thyroiditis. However, diabetic onset in the BB rat is heterogeneous with a subset of the BB rats, which may be genetically distinct, being resistant to diabetes.

3.2.2. Experimentally induced models

Several experimental models have been described which also provide clues to the etiology of IDDM. Streptozotocin (STZ) chemical induced IDDM has been reported, wherein, beta cell destruction is achieved by administration of high doses of selective beta-cell toxic agents such as STZ (19-21). Repeated doses of STZ at sub-diabetogenic doses results in insulitis followed by diabetes which is immunologically mediated. Also, insulitis and diabetes (associated with thyroiditis) can be induced in normal non-autoimmune adult rats by a combination of thymectomy and sublethal irradiation or in athymic rats by transfer of normal spleen cells (22-24). Transgenic mice with genetic manipulations have also provided good animal models for the study of IDDM. Selective beta cell specific expression of various transgenes can be induced, by coupling the transgenes to the insulin gene promoter. Insulitis, the primary characteristic of immunologically mediated diabetes can be induced upon transfer of the SV40 large T antigen in beta cells, late in ontogeny (25). Similar results have been obtained upon transfer of the interferon alpha gene (IFNalpha), tumor necrosis factor (TNF) alpha and interleukin-10 genes (26-29). Mice expressing the major histocompatibility complex (MHC) class I or class II genes and non-MHC molecules such as calmodulin can induce IDDM, though, of a non-immune nature (30-33). IDDM has always been recognized as a hereditary disease and familial transmission of the disease in humans, along with the data from animal models, indicate that IDDM is both polygenic and multifactorial. This has lead to the identification of IDDM susceptibility loci in humans and the NOD mouse model. The studies provide evidence implicating both MHC-linked as well as non-MHC linked genes in the pathogenesis of IDDM (2-4).

3.3. Type II Diabetes or NIDDM

Analogous to IDDM, pathogenesis of NIDDM is an equally complex manifestation of defects in several distinct metabolic functions of insulin and accounts for >90% of patients with diabetes (2,5,6). The main characteristics of NIDDM pathology being (a) peripheral insulin resistance in tissues such as skeletal muscle and adipocytes, leading to inefficient glucose uptake by these organs in response to insulin (b) impaired insulin action to inhibit glucose production by the liver in the face of hyperglycemia and (c) aberrant insulin secretion leading to a decreased insulin output (34). NIDDM is a polygenic disease with a complex inheritance pattern. Moreover, like cancer, the incidence and degree of severity of NIDDM can be exacerbated by the presence of risk factors such as improper diet, lack of physical activity and age. Genetic factors determine the risk of developing NIDDM and susceptibility to insulin resistance and defects in insulin secretion appear to be genetically determined. The evidence of a genetic predisposition in the evolution of a diabetic phenotype is demonstrated by rare mutations in genes encoding glucokinase and transcriptions factors such as the hepatic nuclear factors (HNFs)-1alpha, -1beta and -4alpha, or IPF1, causing maturity onset diabetes in the young (MODY) (35-38).

Most severe forms of Type 2 diabetes occurs due to the inability of the insulin secretory capacity to sufficiently compensate for defects in insulin action. Obesity or excessive weight gain is a major risk factor for the development of Type 2 diabetes. However, all obese people do not develop Type 2 diabetes, due to a capacity of their beta cells to hypersecrete insulin upon demand. Only those obese individuals, who are unable to mount an optimal beta cell compensation response and counter hyperglycemia by increased insulin secretion develop overt diabetes. In agreement with this, it has been observed that patients with Type 2 diabetes have reduced beta cell mass compared to weight-matched non-diabetic individuals (39). A genetically inherited restriction in the ability of beta cells to proliferate may be a factor in the development of Type 2 diabetes in obese individuals. Therefore, a deficient beta cell proliferation capacity can lead to the onset of diabetes or work in conjunction with a risk factor, either a genetic predisposition or an environmental insult, and exacerbate the diabetes pathology.

Table 1. Phenotype of Mice with Alterations in Key Proteins Involved in Insulin Signaling and Diabetes

Altered protein	Approach	Viability	Diabetes Mellitus	Keto-acidosis	Islet Mass	Defects	Ref
Insulin	Knock-out	Yes (death by P2)	Severe	Yes	Hyperplasia	Ketoacidosis, liver steatosis	47
IGF-1	Knock-out	Yes (death after birth or dwarfism)	No	No	ND	Infertility, Dwarfism, defects in musculature, ossification of bones, development of lungs	48, 49
IGF-2	Knock-out	Yes	No	No	ND	Dwarfism	50
IR	Knock-out	Yes (death by one week)	Yes	Yes	ND	Severe diabetes with hyperglycemia and hyperinsulinaemia, liver steatosis, reduced liver glycogen, growth retardation and skeletal muscle defects	51, 52
Beta cell-IR	Cre-LoxP knock-out	Yes	No	No	Reduced in older mice	Loss of insulin secretion in response to glucose, impaired glucose tolerance	53
Muscle-IR	Cre-LoxP knock-out	Yes	No	No	ND	Impaired insulin-stimulated glucose uptake in skeletal muscle, otherwise normal	54
IGF-1R	Knock-out	No	No	No	ND	Death at birth due to respiratory failure, retarded intra-uterine growth, defects in CNS, muscle, bone and skin development	48
IRS-1	Knock-out	Yes	No	No	ND	Relatively normal, mild insulin resistance and post-natal growth retardation	55, 56
IRS-2	Knock-out	Yes	No	Yes	Hypo-plasia	Reduced growth, overt diabetes by 10 weeks, males more affected with early death	57
IR and IRS-1	Double hetero-zygous knock-out	Yes	Yes	No	Hyper-plasia	Double homozygous knock-outs die within 72 hours due to diabetic ketoacidosis, hyperinsulinaemia, insulin resistance	58
Alpha-p85 subunit of PI3K	Knock-out	Yes	No	No	ND	Increased insulin sensitivity, hypoglycemia, increased glucose transport in skeletal muscle and adipocytes	59
GLUT-2	Knock-out	Yes (death by 3 weeks)	Yes (mild)	Yes	Altered develop-ment	Growth retardation and early death, moderate hyperglycemia, hypoinsulinemia due to impaired glucose stimulated insulin secretion, elevated glucagon, altered glucose tolerance,	60
GLUT-4	Knock-out	Yes	No	No	ND	Insulin resistance, impaired glucose and insulin tolerance tests, decreased fat deposition, growth retardation, cardiac hypertrophy, decreased life-span (<7 months)	61
Gluco-kinase	Knock-out	Yes (death by I week)	Yes	Yes	ND	Liver steatosis, high cholesterol and triacylglycerol, depleted liver glycogen	62, 63

4. INSULIN SIGNALING AND ITS COMPONENTS

Insulin was discovered more that 75 years ago which led to expectations that it would result in the rapid amelioration of diabetes. With the realization that discovery of insulin was insufficient to eradicate diabetes, researchers focused their attention on understanding the complex insulin signal transduction network (40-42). This avenue of research was explored with the hope that one or more downstream signaling intermediates of the insulin pathway may be the key to a more complete understanding of insulin biology and may result in an improved awareness of

pathways gone awry in diabetes. Researchers looked for molecules similar to insulin, which led to the discovery of the insulin-like growth factors (IGFs). Studies with insulin and IGFs and characterization of their structure-function motifs led to the important discoveries of the receptors for insulin (IR) and IGF (IGF-R). The IR belongs to a family of growth factor receptors which posses intrinsic tyrosine kinase activity. Upon insulin ligand binding, the IR undergoes auto-phosphorylation of specific tyrosine residues resulting in activation of the receptor kinase. The complex hierarchy of the insulin signaling pathway came unglued with the discovery of key signaling intermediates called the insulin-receptor substrates (41). The active IR phosphorylates tyrosine residues on downstream IRSs, which in turn recruit other proteins like the phosphatidyl inositol 3 (PI3) kinase into the complex allowing signal transduction to proceed to the nucleus. The insulin signaling pathway has been extensively investigated and several comprehensive reviews are available for the interested reader (40-42). The improved knowledge of insulin signaling pathways led to an educated search for genetic alterations of its components in diabetic states. The information gained may offer new therapeutic options for treatment of patients suffering from insulin resistance and other defects of insulin signaling observed in Type 2 diabetes. Naturally occurring mutations of the IR are very rare and the mutations result in a syndrome of extreme insulin resistance such as leprechaunism or the Type A syndrome of insulin resistance and acanthosis nigricans. However, genomic studies have revealed that mutations in the insulin receptor itself do not play a role in the pathology of Type 2 diabetes. IRS-1, the first insulin receptor substrate identified, has several naturally occurring polymorphisms common in Type 2 diabetic patients. Of the several polymorphisms identified to-date, the G972R mutation in IRS-1, is the most common and thereby has been extensively studied in patients as well as *in vitro* (40,43). Obese carriers of this polymorphism exhibit decreased insulin sensitivity during an oral glucose tolerance test. The G972R polymorphism imparts a structural diversity to the IRS-1 molecule since it lies between two putative tyrosine phosphorylation sites involved in binding of the p85 subunit of PI3-kinase and potentially interferes with PI3-kinase and IRS-1 interaction. As expected, expression of the G972R IRS-1 mutant in 32D(IR) cells leads to defective PI3-kinase and IRS-1 binding, leading to a decrease in IRS-1 associated PI3-kinase activity. The mutation does not affect insulin-stimulated IRS-1 tyrosine phosphorylation but the mitogenic effects of insulin are decreased by 35-40%. Although, two polymorphisms in IRS-2 and several in IRS-4 have been detected none have been associated with Type 2 diabetes or insulin resistant states (44,45). A M326I polymorphism of the p85 alpha subunit of PI3-kinase occurs in a region between the SH3 domain and the first SH2 domain of the protein. Individuals homozygous for this mutation exhibit a 32% reduction in insulin sensitivity in an oral glucose tolerance test (46).

5. PHENOTYPE OF MICE WITH ALTERED INSULIN SIGNALING INTERMEDIATES

Expression of several key molecules which comprise the insulin signaling pathway have been altered, either by homologous recombination in embryonic stem (ES) cells to generate mice lacking particular proteins or by generation of transgenic mice expressing increased levels of key proteins (reviewed in 42). Moreover, the specificity of these techniques have been further augmented with the utilization of systems, like the Cre-LoxP system, that allow tissue or cell type specific expression of proteins. Therefore, expression of insulin, insulin receptor (IR), insulin-like growth factor 1 (IGF1), IGF receptor (IGF-R), downstream substrates of insulin signaling like insulin-receptor substrates (IRSs) 1 and 2, the p85-alpha subunit of PI3-kinase, glucose transporters (GLUT2 and GLUT4) as well as key metabolic enzymes such as glucokinase has been altered in mice (table 1). Alterations of several of the above mentioned proteins resulted in phenotypes ranging from mild to no affects of mutations to severe diabetes with associated complications like insulin resistance, ketoacidosis and early demise (47-63). Although, description of phenotypes exhibited by each one of these important studies is not the focus of this review, we present a synopsis of the biological functions attributed to these proteins (table 1).

6. BETA CELL MASS AND DIABETES

There is a constant regulation of insulin synthesis and secretion to meet changes in glucose levels. Defects in any one of the three pronged regulatory mechanisms, viz. insulin synthesis, insulin secretion and changes in beta cell mass, result in a relative or complete insulin deficiency leading to diabetes. Defects in insulin synthesis and secretion have been a focus of extensive research and have been reviewed elsewhere (2,5,6). Although several groups have investigated the regulation of beta cell mass, the current knowledge of important constituents which regulate the capacity of beta cells to proliferate and grow is relatively incomplete. Many of the changes in insulin production are a reflection of changes in total beta cell mass and changes in beta cell mass are important pathological alterations in both types of diabetes. Also, total beta cell mass is increased in response to insulin resistance in obesity, which constitutes a risk factor for development of Type 2 diabetes. In Type 1 diabetes, beta cell mass is depleted due to autoimmune destruction and the remaining beta cells are insufficient in mounting a growth response to counter the increasing hyperglycemia. Although, the pathogenesis of Type 2 diabetes is multifactorial and less well defined, there is increasing evidence that defective beta cell replication and growth may constitute an additive predisposition to the development of the disease. It is likely that in the face of defective beta cell growth, the consequential insulin deficiency diabetes may develop. In support of this hypothesis, it has been observed that the total beta cell mass is decreased in Type 2 diabetes patients compared to weight-matched control subjects (39). Therefore, along with insulin resistance and insulin secretion, defects in beta cell growth should be thoroughly investigated as an important mechanism leading to diabetes.

Beta cell growth is a cumulative effect of the following three phenomena during beta cell development (i) differentiation of beta cells from precursors, a process

referred to as neogenesis (ii) changes in the size of individual beta cells and (iii) replication capacity of existing beta cells (9). The differentiation or neogenesis of beta cells has been extrapolated upon detection of insulin-positive beta cells in pancreatic ducts. There is strong evidence to the existence of neogenesis as a plausible mechanism for changes in beta cell mass based on studies in rat models (64,65). In contrast, changes in size of individual beta cells is not very well documented, even though, glucose, which is the prime stimulator of beta cell replication, increases beta cell size and apparently leads to increased insulin synthesis (66).

Several studies pioneered by Hellerström, Swenne, Weir, Bonner-Weir, Sjöholm, Welsh and Sorenson have lead to an improved understanding of mechanisms associated with beta cell proliferation (7-12,67-69). Swenne performed the initial cell cycle characterization of beta cells and paved the way for further investigations into the replication capacity of beta cells. Islet cell replication has been determined by standard thymidine incorporation assays and more recently using antibody-based bromodeoxyuridine assays.

7. FACTORS REGULATING BETA CELL GROWTH

Beta cell development has been studied extensively and expert reviews are available to the interested reader (67,70). Development of pancreatic endocrine cells in the rat fetus reveals the presence of insulin-positive beta cells by gestational day 13 (71). Measurement of changes in the alpha, beta and delta-endocrine cell population in post-natal rodent islets indicates a continuous increase of beta cell mass throughout post-natal life (72). Morphological quantification of endocrine cells in human fetal pancreas reveals the presence of insulin-positive beta cells by the eighth fetal week (73) with almost a 130-fold increase in beta-cell mass between the 12th week *in utero* and the fifth post-natal month (74). New pancreatic exocrine and islet cells are formed by differentiation of pre-existing embryonic ductal cells, which is referred to as neogenesis, or by replication of beta cells. While neogenesis is the primary mode of increase in beta cell mass during gestation, after birth most of the beta cells are formed by replication.

Studies with rodent islets have been the basis of much of our information of factors influencing beta cell replication. Among the various factors, glucose is a prime regulator of beta cell replication and is known to stimulate replication in both fetal and adult rodent islets (75). In addition, glucose leads to an increased beta cell proliferative compartment (76,77). Insulin and IGF-1 stimulate islet beta cell replication in neonatal rodent pancreatic cells in culture providing evidence that insulin itself can regulate the replication capacity of beta cells in an autocrine fashion (78). This study prompted the examination of several other growth factors for their role in regulating beta cell replication (79). Thus, growth hormone (GH), prolactin and the related placental lactogen, IGF-1, IGF-2 and platelet-derived growth factor (PDGF) have been recognized as stimulators of beta cell replication (80-84). Growth hormone has been reported to stimulate the *in vitro* replication of fetal, neonatal and adult rat beta cells. The stimulation of replication activity resulted in an increased insulin content and secretion where the effects of GH were mimicked by prolactin and its related peptide, placental lactogen.

GH elicits many of its actions by inducing local production of IGFs in target cells. Studies aimed at investigating a similar paracrine pathway operative in islet cells have yielded confusing results. GH, but not glucose, stimulated the release of IGF-1 from fetal and adult rat islets leading to mitogenesis which could be partially negated by addition of monoclonal antibodies to IGF-1 (83,85). The presence of high-affinity IGF-1 receptors on beta cells and the finding that exogenous IGF-1 stimulates beta cell replication (86), supported a concept that GH mitogenic activities might be mediated, at least in part, by a paracrine regulation involving IGF-1. This theory has been challenged by several studies, which failed to demonstrate an intermediary role for IGF-1 in mitogenic activities of GH in beta cells (87). Romanus *et al*., (87) failed to detect increased IGF-1 secretion from islets after GH stimulation while Nielsen *et al*., (reviewed in 8) reported a very modest mitogenic activity for IGF-1, which did not augment the very potent GH stimulatory activity. Other factors which lead to a stimulation of beta cell replication include, amino acids (88), lithium (89), the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA) (90), nicotinamide (91), amniotic fluid (92) and serum (75).

Inhibitors of beta cell proliferation include transforming growth factor beta (TGF-beta), the cytokine interleukin 1-beta (IL1-beta), pancreastatin and the diazepam binding inhibitor, all of which inhibit fetal rodent beta cell proliferation. TGF-beta inhibits glucose stimulated beta cell replication (93). IL1-beta beta suppresses islet cell proliferation in adult mice and rats (94,95). However, the role of IL1-beta in fetal islet cell proliferation is slightly complex with the first 24 hours of stimulation leading to a suppression of beta cell proliferation followed by a potent mitogenic stimulus after 3 days of cytokine exposure. Sjoholm *et al*., identified pancreastatin and diazepam-binding inhibitor (acyl-CoA binding protein) as inhibitors of beta cell replication (96). Both pancreastatin and diazepam-binding inhibitor are produced by islet cells (97-99) and inhibit insulin secretion and may function as inhibitors of beta cell replication *in vivo*.

8. MODELS OF BETA CELL PROLIFERATION IN DIABETES

Since the beta cell is the only source of insulin production, mechanisms responsible for regeneration of beta cells lost or severely reduced in diabetes have been a focus of several studies. These studies have led to the generation and characterization of many animal models, which have yielded important clues regarding the regenerative capacity of beta cells. Beta cell toxins, alloxan or streptozotocin, have been used to selectively destroy beta cells and produce an IDDM-like state. Beta cells, which survive the massive destruction in response to these reagents are capable of replicating, suggesting a replication

capacity in a fraction of beta cells (100,101). The degree of beta cell regeneration, however, is insufficient to cure diabetes (102). Administration of streptozotocin to neonatal rats leads to hyperglycemia due to destruction of beta cells. This is followed by evidence of increased mitotic activity in the surviving beta cells and beta cell neogenesis from undifferentiated precursor cells, resulting in reversion of the hyperglycemia to a normoglycemic state (103-105).

Pancreatectomy, or removal of pancreas, is a very useful approach to demonstrate the regenerative potential of beta cells. 60% partial pancreatectomy does not result in glucose intolerance or permanent diabetes. This maintenance of glucose homeostasis is due to a regeneration among the remaining pancreatic beta cells (106,107). However, when 85-90% partial pancreatectomy is performed, mild hyperglycemia ensues which is followed by increased beta cell replication and a 40% increased beta cell mass (108). Interestingly, 95% pancreatectomy results in severe hyperglycemia with non-existent or very minor signs of beta cell replication (109). Based on the pancreatectomy models, it is evident that beta cells have a certain regenerative capacity. The relative contribution of replication, neogenesis or increased beta cell size to the increased beta cell mass is not very clear at this time. Also, it is likely that the degree of hyperglycemia may dictate the extent of the beta cell replication capacity, with severe hyperglycemia or diabetes negatively affecting the compensatory replication of beta cells. Interestingly, as noted earlier, glucose is one of the stimulators of beta cell replication activity. There is evidence of compensatory growth of beta cells in adult rats after short-term glucose infusion (110). The beta cell growth is due to an increased beta cell hypertrophy and also a five-fold increase in mitotic activity, indicative of beta cell hyperplasia.

The diabetes produced by two single gene mutations, obese (*ob*, chromosome 6), and diabetes (*db*, chromosome 4) vary depending on the genetic background (111). The diabetes is identical, when both the genes are expressed on an inbred genetic background. Increased secretion of insulin, and increased appetite is followed by moderate hyperglycemia with a compensatory increased insulin secretion with an expansion of beta cell mass. When the mutations are expressed on a C57BL/6 inbred background, hypertrophy and hyperplasia of beta cells continues until the hyperglycemia is corrected. However, when the mutations are expressed on the C57BL/Ks background, beta cell expansion in response to the hyperglycemia fails. The consequential islet cell atrophy causes severe hyperglycemia due to deficient insulin secretion leading to uncontrolled diabetes. Islets from C57BL/6 mice with the mutations display higher beta cell replication indices compared to islets isolated from C57BL/Ks mice with mutations. The higher mitotic indices are in response to varying glucose concentrations both *in vitro* and *in vivo* indicating a possible genetically determined lower capacity for beta cell regeneration in the C57BL/Ks strain compared to the C57BL/6 strain (111,112). These inherited genetic differences in the capacity of beta cell replication underlies the predisposition of these two mutant strains to either a severe-obesity, moderate diabetes (C57BL/6 strain) or to a severe life shortening diabetes (C57BL/Ks strain).

There is mounting evidence that defect in beta cell mass is a crucial factor in the progression of a diabetic state in humans. Type 1 or IDDM is a classical example of a low beta cell mass predisposing the onset and progression of diabetes. Continuous destruction of beta cells due an autoimmune attack leads to a severe reduction of beta cell mass. Attempts at regeneration of the beta cell population, via regeneration or replication of the surviving beta cells which escape the immune attack, is met with persistent immune destruction. There is evidence that, Type 2 or NIDDM pathology may also present a condition of deficient beta cell mass associated with the diabetes pathology. Morphometric analysis on autopsy material from human Type 2 diabetics reveals an increased beta cell mass. This increase is due to obesity and an increased insulin resistance which, may lead to a compensatory increase in beta cell mass. Interestingly, carefully controlled studies using both obese as well as non-obese Type 2 patient populations have revealed a definite reduction in beta cell mass compared to age and weight matched non-diabetic subjects (39,113). The low growth rate and the reduced proliferation potential in Type 2 diabetics could be due to defects in growth regulatory proteins. However, studies aimed at identifying the key growth modulatory genes in impairment of beta cell growth and its low proliferation potential have yielded very few clues regarding mechanisms responsible for the deficient beta cell mass in these patients (12,114).

9. CYCLIN/CDK COMPLEXES IN CELL CYCLE

The molecular machinery referred to as the cell cycle clock apparatus, comprising of a diverse set of protein components, orchestrates cellular decisions to proliferate and grow, undergo quiescence, post-mitotic differentiation or apoptosis (figure 1). Changes in cell cycle progression modulate the rate of proliferation and growth. Moreover, the decision made by a cell to exit the cell cycle to undergo an irreversible post-mitotic differentiation state or a state of irreversible cellular senescence is dictated by changes in the cell cycle. Finally, the decision of putting an end to the cellular life-span by undergoing apoptosis is also a reflection of decisions made by proteins regulating the cell cycle machinery (115-117). The cell cycle is typically divided into the following phases, G0 (reversible quiescence), G1 (first gap phase), S (DNA synthesis), G2 (second gap phase) and M (mitosis). Interspersed within the G1-phase is an arbitrary point, referred to as the 'Restriction Point (R)'. At the restriction point, cells which have advanced more than two-thirds of the way through the G1-phase, may decide to commit to an irreversible completion of the cell cycle. Since growth factor stimulation is required only in the first two-thirds part of G1-phase, cells which have committed to pass the restriction point having decided to complete the cell cycle, do not require any additional mitogenic stimulation. On the contrary, if mitogenic stimulation during the first two-thirds of G1-phase has been inappropriate for growth, either due to insufficient positive stimulation (by growth factors like

insulin, IGFs, EGF, PDGF, etc.) or an overwhelming negative stimulation (factors like TGF-beta), cells may elect to re-trace their path to the G0 quiescence state. At this time, cells can enter into a post-mitotic differentiative pathway which in many cases requires cues from distinct growth factor pathways (factors like granulocyte-colony stimulating factor (G-CSF), macrophage-colony stimulating factor (M-CSF), etc.). Therefore, the order and timing of the cell cycle is critical for accurate transmission of genetic information and consequently a number of biochemical pathways have evolved to ensure that initiation of a particular cell cycle event is dependent on the accurate completion of other subsequent events.

The components of the cell cycle machinery is comprised of different protein families which are in turn responsible for the regulated progression of cells through the cell cycle. The core components of the cell cycle clock machinery are the regulatory subunits referred to as the cyclins and their catalytic kinase subunits, the cyclin-dependent kinases (CDKs). Typically, a CDK protein contains a 300 amino acid catalytic domain, which is inactive when it is underphosphorylated and monomeric (121). The primary mechanism of activation is their association with a cyclin partner. Unlike CDKs, which are highly homologous, cyclins are a remarkably diverse family of proteins, ranging in size from about 35 to 90 kDa (115-117). Sequence homology amongst cyclins tends to be concentrated in a 100-residue section known as the cyclin box, which is necessary for CDK binding and activation. In mammalian cells, different cyclin/CDK complexes are assembled and activated at specific points of the cell cycle. Different cyclin-Cdk protein complexes are formed at specific stages of the cell cycle and their activities are required for progression through S phase and mitosis (115-117). At the start of G1-phase, mitogenic growth factors present in serum, or in form of insulin, IGFs, etc. upon receptor binding initiate a cascade of events, chief among them being a rapid and strong induction in expression of cyclin D1. Once synthesized, the D-type cyclins associate with Cdk4 and Cdk6. In mid to late G1 phase, several hours before the onset of the S-phase, cyclin-E is induced which complexes with Cdk2, resulting in cyclin E-Cdk2 complex formation. The activity of this cyclin E-Cdk2 complex seems to be required for the initiation of the S-phase of DNA synthesis. At the onset of DNA synthesis, cyclin A expression is induced, which results in cyclin A-Cdk2 and later cyclin A-Cdc2 (Cdk1) complex formation. This cyclin A-Cdc2 association is maintained until late G2-phase when cyclin B expression is induced. The B-cyclins associate with Cdc2 which trigger complex events associated with mitosis.

9.1. Negative regulation of CDKs

A fully active CDK/Cyclin complex can be turned off by a group of proteins, termed Cyclin Kinase Inhibitors (CKIs) which can bind and inactivate CDK-cyclin complexes (115-117,121). Alternatively, regulatory kinases can phosphorylate the CDK subunit at inhibitory sites near the N-terminus. In mammalian cells, two classes of CKIs, the CIP/KIP and INK4 families, provide tissue-specific mechanisms by which cell cycle progression can be restrained in response to extracellular and intracellular signals. Structure/function analysis of p21 and p27 CIP/KIP proteins show that the N-terminal half of these proteins contain two key domains, one that is required for cyclin binding and the other required for binding to the CDK subunit. The cyclin binding motif appears to be important for providing high-affinity binding and may underlie the specificity of CIP/KIP proteins for G1 cyclin-containing complexes. The INK4 (Inhibitors of Kinase CDK4) family inhibitors associate with CDK4 monomers and reduce the formation of CDK4-Cyclin D complexes *in vivo*. Based on tissue culture studies, it was believed that the primary role for CIP/KIP family of CKIs was the negative regulation of the kinases associated with cyclins D, E, and A. More recent studies have revealed that although the CIP/KIP inhibitors negatively regulate the kinases associated with cyclins E and A, they are positive regulators of the cyclin D-dependent kinases (122,123,124 and references therein). These studies challenge several previous theories and assumptions regarding cell cycle progression and prompt a re-examination of the role of the CIP/KIP proteins in cell cycle control.

D-cyclins act as growth factor sensors and mitogenic stimulation is required for cyclin D transcription, assembly into a CDK complex, transport to the nucleus and eventual ubiquitin-dependent proteasomal degradation. Therefore, the current model for G1-S transition is as follows. Mitogenic signals in the form of either growth factor or cytokine stimulation results in the assembly of cyclin D proteins in complex with members of two distinct families. The cyclin D proteins complex with Cdk4 or Cdk6 kinases and with CIP/KIP inhibitor proteins to form the active cyclin D-Cdk4/6-CIP/KIP complex. Therefore, cyclin D-Cdk4 can sequester CIP/KIP proteins without being inhibited, whereas, activities of CDK2-containing complexes are inhibited by the same CIP/KIP CKIs. Furthermore, studies by LaBaer *et al*., demonstrated that cyclin D-Cdk assembly is facilitated by CIP/KIP proteins (122). These studies demonstrated that, both p21 and p27 promoted complex formation between the cyclin D's and their associated CDKs *in vitro* by stabilizing the complexes. Also, all three CIP/KIP family members, p21,p27 and p57, directed the accumulation of cyclin D-CDK complexes in the nucleus. The addition of a CIP/KIP inhibitor protein into the cyclin D-Cdk4/6 complex results in a reduction of CIP/KIP inhibitor levels free to inhibit the cyclin E-Cdk2 complex. Sequestration of the CIP/KIP inhibitors by the Cyclin D-Cdk4/6 complex thus facilitates activation of the cyclin E-Cdk2 complex in late G1-phase. The association of CIP/KIP inhibitors in complex with cyclin D–Cdk4/6 invites a re-assessment of the role of the other family of CKIs, the INK4 family proteins. There is direct evidence alluding to the role of INK4 family inhibitors in negative regulation of cyclin D-Cdk4/6 complexes. The INK4 family member, p15, mediates G1 arrest induced by TGF-beta (125,126). Also, p16INK4A protein is induced during senescence or aging (127-131), whereas, p18 and p19 have putative roles in differentiation (132-134). Overexpression of INK4 family proteins induces G1-arrest. In light of the evidence that the CIP/KIP inhibitors also bind to CDK4/6 when complexed to cyclin

D proteins, the role of INK family proteins can be assessed as follows. INK4 proteins compete with the CIP/KIP proteins for binding to Cdk4/6. Increased expression of INK4 proteins result in increased cyclin D-Cdk4/6-INK4 complexes and subsequent cyclin D degradation. This leads to a release of CIP/KIP inhibitors from the Cyclin D-Cdk4/6 complex, which now are free to inhibit the cyclin E/A-Cdk2 complex activities. The resultant effect of the concerted action of the INK4 and CIP/KIP inhibitor proteins is G1 phase arrest.

The concerted activation of both the Cyclin D-Cdk4/6-CIP/KIP and cyclin E-Cdk2 complexes results in an ordered phosphorylation of the retinoblastoma family proteins resulting in a relief of E2F repression and transcription of E2F target genes. Among the plethora of key E2F target genes are, the cyclin E and cyclin A proteins, which are necessary to continue cell cycle progression in a forward direction. The newly synthesized cyclin E upon complexing with Cdk2 phosphorylates the p27 CIP/KIP inhibitor and triggers its degradation via a ubiquitin-mediated proteolytic event. The integrated effect of p27 degradation and induction of E2F target genes ensures the progression of the cell cycle which can now proceed irreversibly and without further requirement of a mitogenic stimulus. Finally, degradation of cyclin E, when the cells exit the S-phase, and cyclin A, in G2-phase, returns the system back to ground state. Renewed mitogenic stimulation, which will lead to re-synthesis of D-cyclins, is followed by another turn of the cell cycle.

9.2. Downstream Targets of CDKs

Multiple downstream protein targets have been proposed for the different cyclin/CDK holoenzymes (120). The most studied G1 cyclin/CDK substrate is the product of the retinoblastoma tumor suppressor gene (pRb). pRb is phosphorylated in a cell cycle dependent manner and hypophosphorylated forms of pRb constitute the active forms of this protein (118,119). pRb is hypophosphorylated in quiescent cells and becomes phosphorylated on several CDK consensus sites during mid to late G1. The hypophosphorylated form of pRb binds several cellular proteins and its phosphorylation results in the release of these associated proteins. The cyclin-Cdk complexes are required for progression through the cell cycle, by virtue of their ability to suppress the anti-proliferative effects of the pRb protein which regulate E2F mediated transcription of genes required for S-phase progression (118-120). The ability of pRb to interact with E2F and repress transcription is regulated by phosphorylation catalyzed by Cdks. pRb contains at least 16 consensus sequences for Cdk phosphorylation and it can be phosphorylated by several cyclin-Cdk combinations, including D-cyclins (cyclin D1, D2 and D3) in complex with their kinase counterparts Cdk4 or Cdk6, cyclin E associated with Cdk2 and cyclin A associated with Cdk2 or Cdc2 (Cdk1). Phosphorylation by cyclin D-Cdk4/6 and cyclin E-Cdk2 occurs in G1-phase, whereas cyclin A-Cdk complexes which are not activated until the S-phase add to or maintain the phosphorylation status of pRb during S-phase. Therefore, full hyperphosphorylation of pRb may require a concerted action of multiple cyclin-Cdk complexes.

When it is in its actively growth suppressive state, pRb physically associates with the E2F transcription factors and blocks their ability to activate the expression of genes whose protein products are required for S-phase progression. The ability of pRb to nullify the E2F transcription factors is being investigated extensively and recent evidence indicates that Cdk-mediated phosphorylation events alter interaction of pRb proteins with components of chromatin assembly. Specifically, recent data regarding the regulation of pRb interactions with E2F transcription factors and with proteins like histone-deacetylase (HDAC), mediated by cyclin D-Cdk4/6 as well as cyclin E-Cdk2 provides an interesting mechanism regarding the transcription repression mediated by pRb (135). This paper provides, for the first time, clues regarding the necessity for multiple cyclin-Cdk phosphorylation events that culminate in a fully inactive hyperphosphorylated pRb. Phosphorylation of the C-terminal region of pRb by cyclin D-Cdk4/6 triggers an intermolecular interaction with the pRb pocket region. This interaction inhibits HDAC binding resulting in a block to the active transcriptional repression function of pRb. This initial phosphorylation and loss of HDAC binding facilitates an interaction of the C-terminus with the pocket region. The interaction of the C-terminus with the pRb pocket exposes the S-567 site on pRb for phosphorylation by Cyclin E-Cdk2. This phosphorylation of S-567 results in a disruption of the pRb pocket region that prevents pRb from binding and inactivating E2F. Therefore, in this model the two functions of pRb, (1) active repression and (2) inactivation of E2F, are lost by successive phosphorylation events by cyclin D-Cdk4/6 and then by cyclin E-Cdk2 complexes, respectively. E2F-1 and probably other E2F family members (E2F-2, 3, 4 and 5) along with their heterodimeric partners the DP-1, -2 and -3 proteins, an interaction which is required for the DNA-binding capacity of E2F, bind to pRb, (118). Most of the E2F-responsive genes identified so far are required for G1 transition to S phase of the cell cycle, being transcriptionally activated at a period of G1 phase coincident with passage through the restriction point. The targets for E2F family of transcription factors include Cyclins E and A, CDC2, CDC25 phosphatase, B-*myb*, C-*myc*, N-*myc*, p107 and several of the enzymes involved in DNA metabolism such as DNA polymerase alpha.

10. CELL CYCLE PROGRESSION IN BETA CELLS

Pancreatic beta cells, similar to other cell types, pass through the several distinct phases of the cell cycle. Studies pioneered by Ingemar Swenne and Claes Hellerström have elucidated the replication capacity of beta cells. Swenne maintained beta cell enriched fetal rat pancreatic islets (22 days of gestation) in tissue culture at various glucose concentrations (76). To study the cell cycle and to monitor the effects of glucose on cell cycle progression, beta islet cells were synchronized at the G1/S-phase boundary by incubation in a medium containing 12.5mM hydroxyurea for 24 hours. The rate of cell cycle progression was determined by pulse labeling with ^{3}H-thymidine and after exposure to colchicine, to measure the rate of new beta cell formation. Based on these studies, it

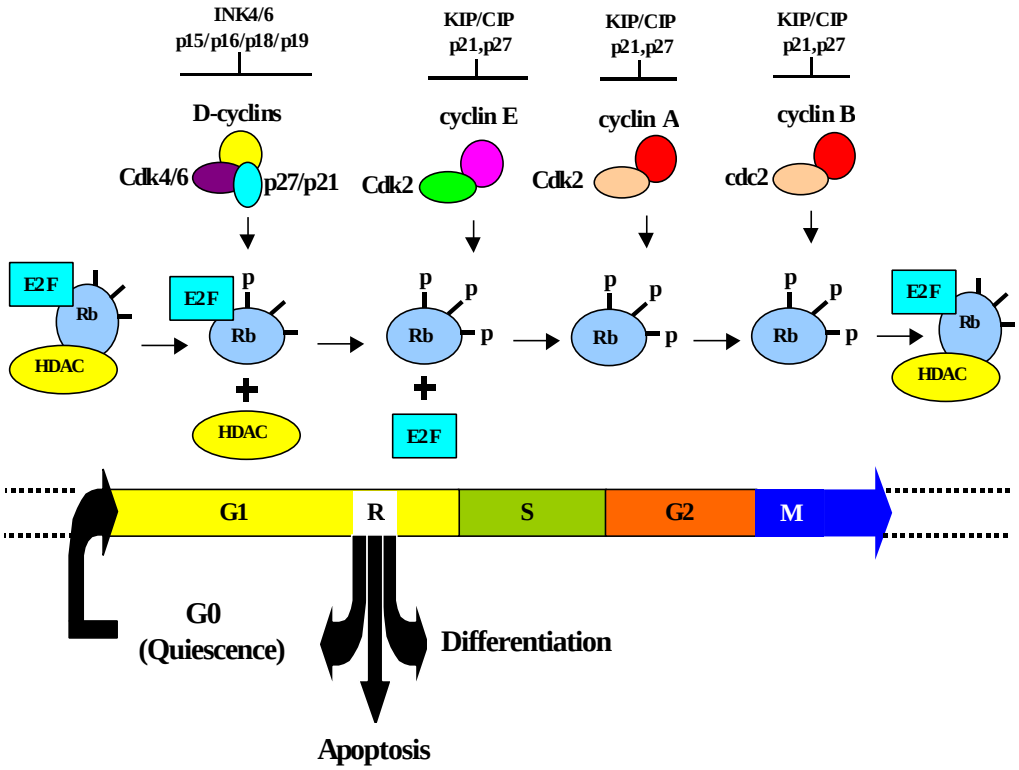

Figure 1. The mammalian cell cycle machinery. Upon receiving stimulatory influences from either cytokines or growth factors, mammalian cells undergo a regulated cell cycle progression. A typical cell cycle is divided into G1, S, G2 and M phases. During progression through the G1 phase, cells pass through 'a restriction point (R)' where cells can leave the cell cycle and enter a reversible quiescence phase (G0), repair damaged DNA, if any and re-enter the cell cycle upon completion of repair, depending on the available mitogenic stimulus. In contrast, in the event of an un-repairable DNA damage scenario, cells can exit the cell cycle at the restriction point and undergo apoptosis. Also, at the restriction point, cells can undergo differentiation, which is an irreversible exit from the cell cycle. During conditions of low mitogenic stimulus, cells exit the cell cycle at the restriction point and undergo quiescence, which can be a reversible process wherein cells can re-enter the cell cycle upon availability of appropriate mitogenic stimulation. In case of cells with a limited life-span, the cell cycle exit is irreversible senescence followed by apoptosis. Cells committed to pass through the cell cycle progress through the S-phase (where DNA synthesis occurs), G2 and M-phase (where cells undergo mitosis). Every phase of the cell cycle is under regulatory influences of different cell cycle proteins. Thus, cyclin D-Cdk4/6 proteins are activated in early G1-phase where the cyclin D-Cdk4/6 assembly and optimal activity requires the p21 and p27 KIP/CIP family proteins. The p21 and p27 proteins inhibit the kinase activities of Cdk2 and cdc2 kinases, complexed with either cyclin E or cyclin A, during late G1, S, G2 and M-phases. In contrast, the p21 and p27 proteins are essential for proper assembly and subsequent activation of the cyclin D-Cdk4/6 complex. The INK4 (INhibitors of Kinase 4) proteins, p15, p16, p18 and p19, inhibit cyclin D-Cdk4/6 kinase activities. The concomitant result of cyclin D-Cdk4/6 activation is phosphorylation of the retinoblastoma (pRb) family or proteins. Phosphorylation of pRb by the cyclin D-Cdk4/6 complex results in a release of histone deacetylase (HDAC) from pRb proteins. Relief of the E2F proteins which occurs after subsequent phosphorylation of pRb by Cyclin E-Cdk2 leads to release of bound E2F resulting in increased expression of genes required for DNA synthesis during S-phase. The pRb proteins are maintained in their hyperphosphorylated state upon subsequent phosphorylation by cyclin A-Cdk2 and cyclin B-Cdk2 or Cdc2 (Cdk1) complexes. This maintenance of pRb proteins in hyper-phosphorylated state precludes the necessity for additional mitogenic stimulation to ensure completion of the cell cycle. Finally, the return of the pRb proteins back to their hypo-phosphorylated states, which leads to re-sequestration of the HDAC and E2F proteins, takes the cell cycle back to ground state. Further cell cycle turns will require resumption of mitogenic stimulation and re-activation of the cell cycle kinases.

was proposed that the time for a full cell cycle was 14.9 hours, which could be further sub-divided into G1-phase (2.5 hours), S-phase (6.4 hours), G2-phase (5.5 hours) and M-phase (0.5 hour). Glucose stimulated an increase in proliferation of beta cells, however, the progression of beta cells through the cell cycle was similar at differing glucose concentrations. These observations prompted two inferences, (a) glucose stimulated beta cell proliferation by increasing the number of cells entering the cell cycle and (b) only a limited fraction of the total beta cell population is capable of entering the active cell cycle. Furthermore, these studies allowed an estimation of the rate of new beta cell

formation per 24 hours, which indicated that 4.2% new beta cells were formed in the presence of 2.7 mM glucose, whereas, 10.4% new beta cells were formed in the presence of 16.7 mM glucose. Furthermore, an age-dependent study of cell cycle progression of beta cells isolated from fetal, 1-week, 3-week and 3-month old rats revealed that the cell cycle was similar in all age groups (77). When the glucose concentration was increased from 2.7 to 16.7 mM, the rate of beta cell proliferation increased 2.5-fold in all age-groups, but there was no further increase in proliferation at 33 mM glucose indicating that high concentrations of glucose may inhibit beta cell proliferation. Also, at any glucose concentration, the rate of beta cell formation decreased with increasing donor age. Careful analysis revealed that only a fraction of islet cells retained a capacity of entering the cell cycle and undergoing mitosis. 10% fetal islet beta cells were capable of entering the cell cycle and this percentage was reduced to less that 3% in adult islet beta cells. Islet cell thymidine kinase (TK) activity was also measured as an indicator of beta cell proliferation in the rat pancreas (136). TK activity was preferentially increased during the S-phase of the cell cycle and the activity in freshly isolated islets declined with the age of the animal.

The growth of beta cells is determined by the number of beta cells entering the cell cycle rather than changes in the rate of the cycle. The beta cell passes through the cell cycle at a relatively high rate but the fraction of proliferating cells is low. During fetal life, the beta cell exhibits a poor insulin response to glucose. In late fetal life, glucose is a strong stimulus to beta cell replication and the metabolism of glucose is a pre-requisite for this process. Glucose stimulates proliferation by recruiting beta cells from a resting G0 state, into the proliferative compartment composed of cells in an active cell cycle. However, the proliferative compartment comprises <10% of the total islet cell population even at maximal glucose stimulation. However, this ~10% of beta cells in the active cell cycle is reduced as a fraction of age, where only about 3% of the total adult beta cells are capable of entering the active cell cycle. This drastic reduction of beta cell proliferation with increasing age is, most likely, due to a gradual withdrawal of cells from the active cell cycle into an irreversible G0 state. However, the observation that a very small fraction of beta cells are capable of entering the cell cycle argues that beta cells have replication potential. This fraction can be potentially increased by recruitment of beta cells, which are in the quiescent G0 phase to re-enter the cell cycle and undergo replication.

The notion of a limited pool of beta cells capable of entering the cell cycle was questioned by Brelje *et al*., who studied the regulation of islet beta cell proliferation in response to prolactin (PRL) (69). Insulin secretion and beta cell proliferation increased significantly in neonatal rat islets in response to prolactin. Initial PRL mitogenic stimulus occurred by a limited procurement of non-dividing beta cells into the cell cycle followed by majority of the daughter cells proceeding directly into additional cell division cycles. The maximal PRL stimulatory affect was maintained by a continued high rate of recruitment of beta cells into the cell cycle with only about one-fourth of the daughter cells continuing to divide. This study suggested that instead of a limited pool of beta cells capable of cell division, beta cells are transiently entering the cell cycle and dividing infrequently in response to PRL, indicating that the majority of beta cells are not in an irreversible G0-phase. This observation partly contradicts the initial islet cell cycle studies and prompts a careful analysis of the cell cycle machinery active in beta cells.

The re-entry of resting beta cells into the active cell cycle requires the knowledge of proteins involved in regulation of cell cycle progression of beta cells. At this time, we have very little knowledge of the molecules which determine the cell cycle kinetics of beta cells. The low proliferative capacity of beta cells has also been proposed to result from a low expression of p34CDC2 Serine/threonine kinase and cyclin B1 which are necessary for normal progression of the cell cycle (114).

Several other studies highlight the role of cell cycle proteins in controlling the replication capacity of beta cells. Expression of growth promoting genes such as SV40 large T antigen and the oncogenes *v-src*, *myc* and *ras* have been altered either in transgenic mice or in islet cells in culture. Transgenic mice with insulin promoter driven beta cell specific expression of SV40 large T antigen developed insulinomas (137). However, the observation that expression of large T antigen was not sufficient to form beta cell tumors indicated that transformation of beta cells is a rare event requiring multiple co-operating mutations. Beta cells derived from the SV40 large T antigen transgenic mice maintained elevated DNA synthetic rates compared to control islets in which the DNA synthesis gradually decreased with age (138). Beta cells from transgenic mice harbored elevated levels of p53 protein, which can bind to SV40 large T antigen. The interaction is thought to inactivate the anti-proliferative activity of p53 (139,140).

Transfection of activated *v-src* oncogene, a cytoplasmic tyrosine kinase, into beta cells stimulated DNA synthesis and substrate phosphorylation (141). Similarly, transfection of activated *myc* and *ras* oncogenes also led to increased rates of DNA synthesis (141). DNA synthesis in beta cells was also stimulated by overexpression of growth factor receptors such as the platelet-derived growth factor receptor (PDGF-R) and fibroblast growth factor receptor (FGF-R). Growth factor mediated signal transduction pathways lead to changes in expression of cell cycle proteins, eventually, resulting in the increased proliferation effects.

Identification of cell cycle modulators of beta cell proliferation will provide insights into the replication potential of fetal, young and adult islet cells. Moreover, this knowledge can then be utilized to explore if any one or more of these proteins can be targeted to achieve an increased replication competence in a previously resting beta cell. Identification of such factors will open new avenues towards understanding beta cell biology and will

allow a modulation of beta cell replication potential in Type 1 and Type 2 diabetes. If this enriched beta cell pool can be rendered immuno-previledged with other distinct genetic manipulations, restoration of the beta cell population in Type 1 or IDDM may become feasible.

11.Cdk4 DETERMINES BETA CELL PROLIFERATION POTENTIAL

We propose a model for regenerating the beta cell replication potential based on results obtained from studies using two mouse strains harboring mutations in cyclin-dependent kinase 4 (Cdk4), a cell cycle kinase (142). We targeted the mouse *Cdk4* locus by homologous recombination to generate two strains of mice, one that lacks Cdk4 expression, *Cdk4*$^{neo/neo}$, and the other that expresses an activated Cdk4, *Cdk4*$^{R24C/R24C}$. *Cdk4*$^{neo/neo}$ mice are viable, but small in size and infertile. The tissue-specific importance of Cdk4 was underscored with the observation that adult *Cdk4*$^{neo/neo}$ mice exhibit diabetic characteristics such as polyuria, polydipsia and impaired locomotion (2).

It is interesting that disruption of Cdk4, which results in absence of detectable Cdk4 protein does not result in a lethal phenotype. Based on several results it could have been anticipated that loss of Cdk4 may be lethal to the growth and proliferation of cells of diverse origin. This finding is similar to that observed in mice with disruption of Cyclin D1 and Cyclin D2 genes (143,144). Cyclin D1 mutant mice are viable but severely reduced in size and life-span (143). Moreover, they display tissue-specific abnormalities, namely : neurological impairment, hypoplastic retinas and a failure to undergo normal mammary epithelial development during pregnancy. Cyclin-D2 deficient mice are also viable with no defects in overall growth (144). However, Cyclin D2 deficient female mice are sterile owing to the inability of the ovarian granulosa cells to proliferate normally in response to follicle-stimulating hormone (FSH), whereas mutant males display hypoplastic testes. These observations highlight the apparent tissue-specific nature of regulation mediated by the two cyclin D proteins. Defects in Cyclin D1 mutant mice described above, can be corrected by replacement of Cyclin E in the Cyclin D1 deficient mouse background (145). The replacement of Cyclin E rescues all phenotypic abnormalities due to loss of Cyclin D1, restoring normal development to all affected tissues (145). This study indicates that cyclin E may be the major target of cyclin D1 and activation of cyclin E may be the primary function of Cyclin D1-Cdk4/6 proteins. *Cdk4*$^{neo/neo}$ mice are reduced in growth and infertile, a phenotype seen also in Cyclin D1 and D2 mutant mice, respectively. Whether, the phenotypes observed in the *Cdk4*$^{neo/neo}$ and *Cdk4*$^{R24C/R24C}$ (see below) mice are a reflection of changes in Cyclin E associated activities is, at this time, not clear.

Insulin levels in serum of *Cdk4*$^{neo/neo}$ mice were reduced by 90% and serum glucose levels were increased by 2 to 3 fold. Consistent with these findings, *Cdk4*$^{neo/neo}$ mice had very high levels of glucose and ketone bodies in their urine. Interestingly, there were no appreciable differences in insulin levels between 2 and 8 month old *Cdk4*$^{neo/neo}$ mice, whereas, 8 month old *Cdk4*$^{neo/neo}$ mice had higher levels of serum glucose, indicating moderate insulin resistance with increasing age. The above results suggested that loss of Cdk4 expression might affect insulin production in *Cdk4*$^{neo/neo}$ mice. Histological analysis of pancreatic tissue revealed a severe deformity and a dramatic reduction in the size and number of their islets. To substantiate these results, we performed morphometric analysis using pancreatic tissue obtained from *Cdk4*$^{neo/neo}$ mice and their wild-type controls. The islet area in *Cdk4*$^{neo/neo}$ mice, is reduced by 13-15 fold in comparison to islets from control littermates. As a consequence, the islet:pancreas and islet:acinar ratios are decreased whereas the acinar:islet ratios are elevated in the *Cdk4*$^{neo/neo}$ mice. These observations indicate a selective developmental defect in the endocrine islet compartment of the *Cdk4*$^{neo/neo}$ pancreas. These observations were confirmed by immunohistochemical analysis using antibodies specific for insulin, where a severe reduction in insulin immunoreactivity was observed. Normal, or close to normal levels of glucagon and somatostatin producing islet cells were observed in these mutant mice. These results indicate that loss of Cdk4 expression in these mice specifically affects beta islet cells. The combined phenotype of insulin deficient diabetes, decreased growth and infertility seen in *Cdk4*$^{neo/neo}$ mice could be due to defective insulin production since disruption of components of the insulin signaling pathway also results in infertility and reduced growth (47-52,55-58).

Our results indicate that the diabetic condition observed in these mice resembles very closely the IDDM observed in humans. However, we have not observed evidence of an autoimmune component in the reduction of beta cell mass, a process widely believed to be the cause of beta cell destruction in IDDM of humans.

To verify the role of Cdk4 in beta cell development, diabetes as well as to determine the consequence of Cdk4 over-activation on mouse development, we generated mice expressing a mutant Cdk4 protein that cannot be down regulated by the cell cycle inhibitor P16INK4a (146,147). This mutation was expected to result in the expression of an activated Cdk4 protein, which would allow a comparison with the *Cdk4*$^{neo/neo}$ mice, which lack Cdk4 expression. *Cdk4*$^{R24C/R24C}$ mice do not display any characteristics observed in diabetic *Cdk4*$^{neo/neo}$ mice. Instead, the major abnormality observed in these mice is a dramatic hyperplasia in their pancreatic islets resembling insulinomas. Morphometric analysis indicate that the islet area in *Cdk4*$^{R24C/R24C}$ mice is increased by 7-10 fold in comparison to that observed in control littermates. As a consequence, the islet:pancreas and islet:acinar ratios are increased whereas the acinar:islet ratios are severely reduced. These observations indicate a preferential hyperplasia of the islet compartment of the *Cdk4*$^{R24C/R24C}$ pancreas. Immunohistochemical staining revealed that these hyperplastic islets were primarily made of beta cells

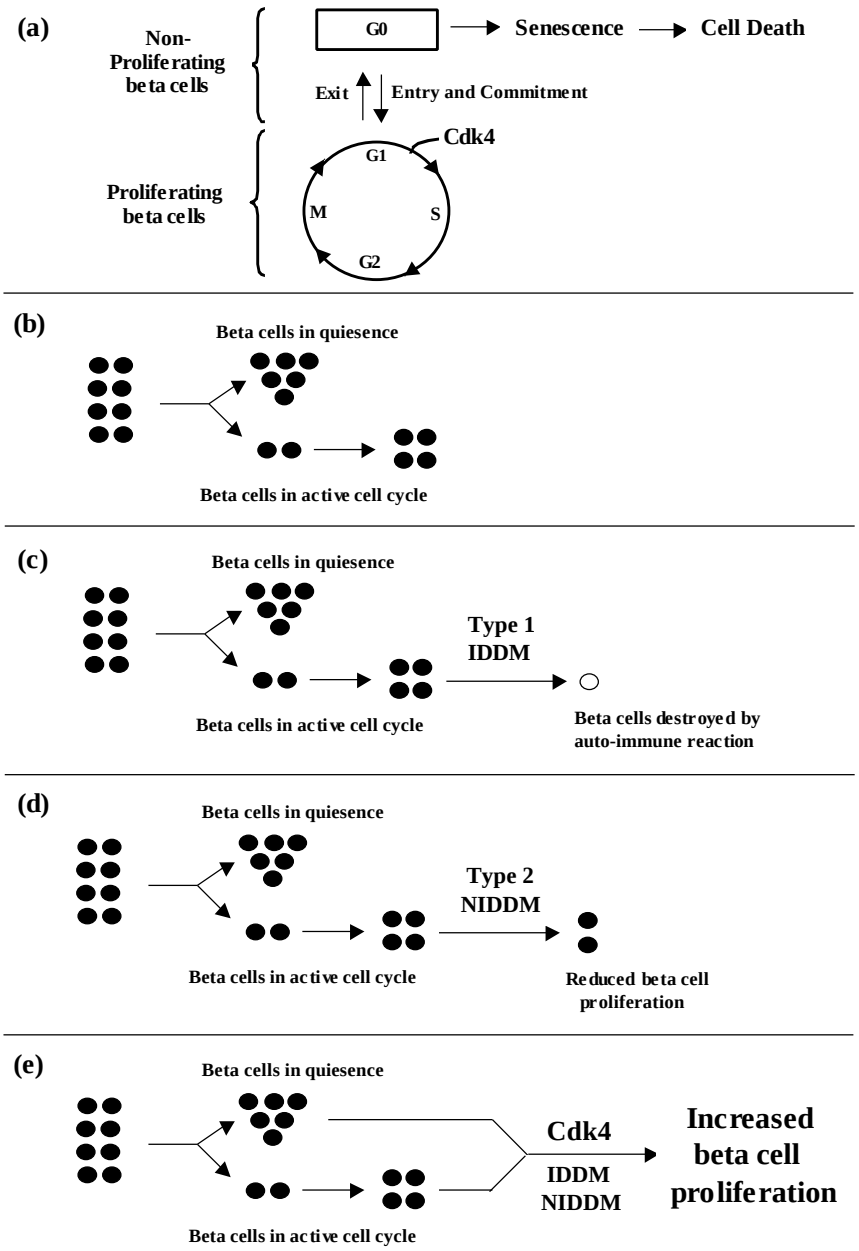

Figure 2. Model for beta cell rescue by modulation of beta cell regeneration potential by Cdk4. (a) Beta cells, like other cell types, progress through the four identifiable stages of the cell cycle, G1 where cells can make a decision to exit the cell cycle and enter a quiescence (G0) state. In G0, beta cells do not replicate DNA, but are in a quiescence state and can re-enter the cell cycle. A majority of beta cells are in a G0 state and many of them undergo senescence followed by cell death. A very small fraction of cells which retain the capacity for cell division, can re-enter the cell cycle from G0 upon expression of G1 Cyclins and Cdks. An important regulator of this commitment to progress through the G1-phase is Cdk4. Once beta cells enter the cell cycle they go through the other phases, S, where DNA synthesis occurs followed by G2 and M-phases where the cells undergo division. (b) Beta cells retain a relatively low capacity of cell division and majority of beta cells are in G0 or quiescent state. A very small fraction of beta cells have a potential to divide. (c) In Type 1 diabetes, the already low fraction of beta cells which have a cell division capacity are targeted for autoimmune destruction leading to severe beta cell atrophy and diabetes. (d) In patients with Type 2 diabetes, reduced beta cell proliferation may add to the pathology of the disease i.e. insulin resistance in target tissues and defects in insulin secretion. (e) Our results indicate that Cdk4 regulates the proliferative capacity of beta cells. We propose that an increase of Cdk4 activity in beta cells of IDDM patients may compensate the loss of beta cells to autoimmune destruction and allow a re-population of the beta cell compartment. Pathology of NIDDM may also predispose an inherited or acquired deficiency in beta cell proliferation which may involve changes in Cdk4 activity.

since they were highly immunoreactive for insulin. No significant differences were observed in the levels of glucagon and somatostatin immunoreactive cells indicating that most of the hyperplastic islets were made of beta cells. Semi-quantitative RT-PCR analysis confirmed that beta cell-specific transcripts were significantly increased. These observations illustrate the critical and specific role that Cdk4 activity plays in pancreatic beta cell development and proliferation.

12. PERSPECTIVE

The discovery of insulin more than 75 years ago fueled enthusiastic optimism regarding insulin therapy of diabetes. However, to this day complications of diabetes still produce devastating consequences and it is believed that better control of glucose levels will reduce the rate and severity of these complications. Although, insulin therapy is now better due to the availability of insulin pumps and automated glucose monitoring, only a small portion of patients with IDDM obtain sufficient glycemic control. In lieu and in conjunction with insulin therapy, several alternate modes of therapy are under investigation. While many of them are very unique and interesting concepts, all of the procedures so far have met with limited success. Pancreatic and islet transplantation approaches have been experimented upon with the goal of providing exogenous sources for insulin. In animal models of IDDM, pancreatic and islet transplantation has been successful in establishing euglycemia. The successful isolation of pancreatic islets by Lacy and Kostianovsky in 1967 and later by Hellerström *et al*., opened the door for islet transplantation as a mode for treatment of diabetes (148,149). Two feasible routes for such a therapy are (1) beta-cell transplantation and (2) a mechanical beta cell. Both of these avenues are currently under investigation and offer hopes for a treatment option for diabetes (reviewed in 150). The first successful islet transplants were performed in rodents in the 1970s, but unfortunately, very few human diabetic patients have received any benefit from beta cells replacement therapy. Isl*et al*lografts as a mode of therapy offer a theoretically convenient approach since islets can be delivered to the liver via the portal vein with a relatively simple procedure. However, the initial success rate of this procedure has been very low. The two major problems facing islet transplantation being (1) finding a satisfactory source of insulin producing cells and (2) how can the transplanted cells be protected form destruction by the immune system through the processes of autoimmunity and transplant rejection. The source for insulin producing tissue has been from either pancreatic tissue from cadaver donors or half of the pancreas from living donors. Both of these approaches have potential drawbacks, the most striking of which is the lack of supply of available pancreatic tissue and also the possibility of inducing a diabetic state in living pancreatic tissue donors, due to a reduction in their beta cell mass. Use of fetal or neonatal pancreatic tissue, which provides an attractive source due to its increased growth potential as well as xeno-transplantation approaches with tissues from pigs have been explored with limited success.

Efforts to expand beta cells and create insulin-producing cells with genetic engineering offer an attractive option for therapy. Although beta cells have capacity for some growth, it has not been possible to efficiently expand beta cells in the laboratory. Efforts are underway to create beta cell lines that might be useful for transplantation. By adding additional genes that influence glucose metabolism, it may be possible to manipulate these cells so they secrete insulin when exposed to glucose. These genes can be transferred into beta cells by genetic approaches. The transplanted islets can be protected from transplant rejection or autoimmune attack by similar genetic modifications, which may help the beta cell to escape recognition by the immune system. Knowledge about genes necessary to replicate and increase the division potential of these beta cells will definitely aid these process of genetic manipulation of beta cells and a breakthrough allowing expansion of human beta cells would solve the supply problem for transplantable insulin-producing cells.

Our results provide a basis for a model for alteration of cell cycle pathways to modify the proliferation potential of beta cells and regenerate beta cells lost or severely depleted in diabetes (figure 2). This information is crucial since when it come to insulin production from beta cells *"it is a numbers game"*!

13. REFERENCES

1. Rotter J. I, Vadheim C. M, Rimoin D. L. Genetics of diabetes mellitus. In Rifkin H, Porte D (eds) Diabetes Mellitus. Theory and Practice, ed 4. Elsevier, Amsterdam, pp 378-413 (1990)
2. F. M. Ashcroft and S. J. H. Ashcroft, Insulin: Molecular Biology to Pathology, (Oxford University Press, New York, 1992); R. H. Unger and D. W. Foster,Williams Textbook of Endocrinology, J. D. Wilson and D. W. Foster, Eds. (Saunders, Philadelphia, 1985), p. 1018 (1992)
3. Bach, J. F. : Insulin-dependent diabetes mellitus as an autoimmune disease. *Endocr Rev* 15, 516-42 (1994)
4. Tisch, R., and McDevitt, H. : Insulin-dependent diabetes mellitus. *Cell* 85, 291-7 (1996)
5. Taylor, S. I. : Deconstructing type 2 diabetes. *Cell* 97, 9-12 (1999)
6. Kahn, B. B. : Type 2 diabetes: when insulin secretion fails to compensate for insulin resistance. *Cell* 92, 593-6 (1998)
7. Bonner-Weir, S. : Regulation of pancreatic beta-cell mass in vivo. *Recent Prog Horm Res* 49, 91-104 (1994)
8. Sjoholm, A. : Intracellular signal transduction pathways that control pancreatic beta- cell proliferation. *FEBS Lett* 311, 85-90 (1992)
9. Swenne, I. : Pancreatic beta-cell growth and diabetes mellitus. *Diabetologia* 35, 193-201 (1992)
10. Sjoholm, A. : Diabetes mellitus and impaired pancreatic beta-cell proliferation. *J Intern Med* 239, 211-20 (1996)
11. Hellerstrom, C., and Swenne, I. : Functional maturation and proliferation of fetal pancreatic beta-cells. *Diabetes* 40 Suppl 2, 89-93 (1991)
12. Welsh, M., Mares, J., Oberg, C., and Karlsson, T. : Genetic factors of importance for beta-cell proliferation. *Diabetes Metab Rev* 9, 25-36 (1993)
13. Makino, S., Kunimoto, K., Muraoka, Y., Mizushima, Y., Katagiri, K., and Tochino, Y. : Breeding of a non-

obese, diabetic strain of mice. *Jikken Dobutsu* 29, 1-13 (1980)
14. Atkinson, M. A., and Leiter, E. H. : The NOD mouse model of type 1 diabetes: as good as it gets? *Nat Med* 5, 601-4 (1999)
15. Harada, M., and Makino, S.: Promotion of spontaneous diabetes in non-obese diabetes-prone mice by cyclophosphamide. *Diabetologia* 27, 604-6 (1984)
16. Matsumoto, M., Yagi, H., Kunimoto, K., Kawaguchi, J., Makino, S., and Harada, M. : Transfer of autoimmune diabetes from diabetic NOD mice to NOD athymic nude mice: the roles of T cell subsets in the pathogenesis. *Cell Immunol* 148, 189-97 (1993)
17. Christianson, S. W., Shultz, L. D., and Leiter, E. H. : Adoptive transfer of diabetes into immunodeficient NOD-scid/scid mice. Relative contributions of CD4+ and CD8+ T-cells from diabetic versus prediabetic NOD.NON-Thy-1a donors. *Diabetes* 42, 44-55 (1993)
18. Nakhooda, A. F., Like, A. A., Chappel, C. I., Murray, F. T., and Marliss, E. B. : The spontaneously diabetic Wistar rat. Metabolic and morphologic studies. *Diabetes* 26, 100-12 (1977)
19. Like, A. A., and Rossini, A. A. : Streptozotocin-induced pancreatic insulitis: new model of diabetes mellitus. *Science* 193, 415-7 (1976)
20. Kolb, H. : Mouse models of insulin dependent diabetes: low-dose streptozocin- induced diabetes and nonobese diabetic (NOD) mice. *Diabetes Metab Rev* 3, 751-78 (1987)
21. Paik, S. G., Fleischer, N., and Shin, S. I. : Insulin-dependent diabetes mellitus induced by subdiabetogenic doses of streptozotocin: obligatory role of cell-mediated autoimmune processes. *Proc Natl Acad Sci U S A* 77, 6129-33 (1980)
22. Fowell, D., and Mason, D. : Evidence that the T cell repertoire of normal rats contains cells with the potential to cause diabetes. Characterization of the CD4+ T cell subset that inhibits this autoimmune potential. *J Exp Med* 177, 627-36 (1993)
23. Stumbles, P. A., and Penhale, W. J. : IDDM in rats induced by thymectomy and irradiation. *Diabetes* 42, 571-8 (1993)
24. McKeever, U., Mordes, J. P., Greiner, D. L., Appel, M. C., Rozing, J., Handler, E. S., and Rossini, A. A. : Adoptive transfer of autoimmune diabetes and thyroiditis to athymic rats. *Proc Natl Acad Sci U S A* 87, 7618-22 (1990)
25. Adams, T. E., Alpert, S., and Hanahan, D. : Non-tolerance and autoantibodies to a transgenic self antigen expressed in pancreatic beta cells. *Nature* 325, 223-8 (1987)
26. Stewart, T. A., Hultgren, B., Huang, X., Pitts-Meek, S., Hully, J., and MacLachlan, N. J. : Induction of type I diabetes by interferon-alpha in transgenic mice. *Science* 260, 1942-6 (1993)
27. Higuchi, Y., Herrera, P., Muniesa, P., Huarte, J., Belin, D., Ohashi, P., Aichele, P., Orci, L., Vassalli, J. D., and Vassalli, P. : 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, 1719-31 (1992)
28. Picarella, D. E., Kratz, A., Li, C. B., Ruddle, N. H., and Flavell, R. A. : Transgenic tumor necrosis factor (TNF)-alpha production in pancreatic islets leads to insulitis, not diabetes. Distinct patterns of inflammation in TNF-alpha and TNF-beta transgenic mice. *J Immunol* 150, 4136-50 (1993)
29. Wogensen, L., Huang, X., and Sarvetnick, N. : Leukocyte extravasation into the pancreatic tissue in transgenic mice expressing interleukin 10 in the islets of Langerhans. *J Exp Med* 178, 175-85 (1993)
30. Allison, J., Campbell, I. L., Morahan, G., Mandel, T. E., Harrison, L. C., and Miller, J. F. : Diabetes in transgenic mice resulting from over-expression of class I histocompatibility molecules in pancreatic beta cells. *Nature* 333, 529-33 (1988)
31. Markmann, J., Lo, D., Naji, A., Palmiter, R. D., Brinster, R. L., and Heber-Katz, E. : Antigen presenting function of class II MHC expressing pancreatic beta cells. *Nature* 336, 476-9 (1988)
32. Gotz, J., Eibel, H., and Kohler, G. : Non-tolerance and differential susceptibility to diabetes in transgenic mice expressing major histocompatibility class II genes on pancreatic beta cells. *Eur J Immunol* 20, 1677-83 (1990)
33. Epstein, P. N., Overbeek, P. A., and Means, A. R. : Calmodulin-induced early-onset diabetes in transgenic mice. *Cell* 58, 1067-73 (1989)
34. Kahn, C. R., Vicent, D., and Doria, A. : Genetics of non-insulin-dependent (type-II) diabetes mellitus. *Annu Rev Med* 47, 509-31 (1996)
35. Yamagata, K., Furuta, H., Oda, N., Kaisaki, P. J., Menzel, S., Cox, N. J., Fajans, S. S., Signorini, S., Stoffel, M., and Bell, G. I. : Mutations in the hepatocyte nuclear factor-4alpha gene in maturity- onset diabetes of the young (MODY1) [see comments]. *Nature* 384, 458-60 (1996)
36. Yamagata, K., Oda, N., Kaisaki, P. J., Menzel, S., Furuta, H., Vaxillaire, M., Southam, L., Cox, R. D., Lathrop, G. M., Boriraj, V. V., Chen, X., Cox, N. J., Oda, Y., Yano, H., Le Beau, M. M., Yamada, S., Nishigori, H., Takeda, J., Fajans, S. S., Hattersley, A. T., Iwasaki, N., Hansen, T., Pedersen, O., Polonsky, K. S., Bell, G. I., and *et al*. : Mutations in the hepatocyte nuclear factor-1alpha gene in maturity- onset diabetes of the young (MODY3) [see comments]. *Nature* 384, 455-8 (1996)
37. Stoffers, D. A., Ferrer, J., Clarke, W. L., and Habener, J. F. : Early-onset type-II diabetes mellitus (MODY4) linked to IPF1 [letter]. *Nat Genet* 17, 138-9 (1997)
38. Horikawa, Y., Iwasaki, N., Hara, M., Furuta, H., Hinokio, Y., Cockburn, B. N., Lindner, T., Yamagata, K., Ogata, M., Tomonaga, O., Kuroki, H., Kasahara, T., Iwamoto, Y., and Bell, G. I. : Mutation in hepatocyte nuclear factor-1 beta gene (TCF2) associated with MODY [letter]. *Nat Genet* 17, 384-5 (1997)
39. Kloppel, G., Lohr, M., Habich, K., Oberholzer, M., and Heitz, P. U. : Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. *Surv Synth Pathol Res* 4, 110-25 (1985)
40. Virkamaki, A., Ueki, K., and Kahn, C. R. : Protein-protein interaction in insulin signaling and the molecular mechanisms of insulin resistance. *J Clin Invest* 103, 931-43 (1999)
41. White, M. F. : The IRS-signalling system: a network of docking proteins that mediate insulin action. *Mol Cell Biochem* 182, 3-11 (1998)
42. Lamothe, B., Baudry, A., Desbois, P., Lamotte, L., Bucchini, D., De Meyts, P., and Joshi, R. L. : Genetic

engineering in mice: impact on insulin signalling and action. *Biochem J* 335, 193-204 (1998)
43. Almind, K., Inoue, G., Pedersen, O., and Kahn, C. R. : A common amino acid polymorphism in insulin receptor substrate-1 causes impaired insulin signaling. Evidence from transfection studies. *J Clin Invest* 97, 2569-75 (1996)
44. Kalidas, K., Wasson, J., Glaser, B., Meyer, J. M., Duprat, L. J., White, M. F., and Permutt, M. A. : Mapping of the human insulin receptor substrate-2 gene, identification of a linked polymorphic marker and linkage analysis in families with Type II diabetes: no evidence for a major susceptibility role. *Diabetologia* 41, 1389-91 (1998)
45. Almind, K., Frederiksen, S. K., Ahlgren, M. G., Urhammer, S., Hansen, T., Clausen, J. O., and Pedersen, O. : Common amino acid substitutions in insulin receptor substrate-4 are not associated with Type II diabetes mellitus or insulin resistance. *Diabetologia* 41, 969-74 (1998)
46. Hansen, T., Andersen, C. B., Echwald, S. M., Urhammer, S. A., Clausen, J. O., Vestergaard, H., Owens, D., Hansen, L., and Pedersen, O. : Identification of a common amino acid polymorphism in the p85alpha regulatory subunit of phosphatidylinositol 3-kinase: effects on glucose disappearance constant, glucose effectiveness, and the insulin sensitivity index. *Diabetes* 46, 494-501 (1997)
47. Duvillie, B., Cordonnier, N., Deltour, L., Dandoy-Dron, F., Itier, J. M., Monthioux, E., Jami, J., Joshi, R. L., and Bucchini, D. : Phenotypic alterations in insulin-deficient mutant mice. *Proc Natl Acad Sci U S A* 94, 5137-40 (1997)
48. Liu, J. P., Baker, J., Perkins, A. S., Robertson, E. J., and Efstratiadis, A. : Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell* 75, 59-72 (1993)
49. Powell-Braxton, L., Hollingshead, P., Warburton, C., Dowd, M., Pitts-Meek, S., Dalton, D., Gillett, N., and Stewart, T. A. : IGF-I is required for normal embryonic growth in mice. *Genes Dev* 7, 2609-17 (1993)
50. DeChiara, T. M., Efstratiadis, A., and Robertson, E. J. : A growth-deficiency phenotype in heterozygous mice carrying an insulin- like growth factor II gene disrupted by targeting. *Nature* 345, 78-80 (1990)
51. Accili, D., Drago, J., Lee, E. J., Johnson, M. D., Cool, M. H., Salvatore, P., Asico, L. D., Jose, P. A., Taylor, S. I., and Westphal, H. : Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. *Nat Genet* 12, 106-9 (1996)
52. Joshi, R. L., Lamothe, B., Cordonnier, N., Mesbah, K., Monthioux, E., Jami, J., and Bucchini, D. : Targeted disruption of the insulin receptor gene in the mouse results in neonatal lethality. *Embo J* 15, 1542-7 (1996)
53. Kulkarni, R. N., Bruning, J. C., Winnay, J. N., Postic, C., Magnuson, M. A., and Kahn, C. R. : Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. *Cell* 96, 329-39 (1999)
54. Bruning, J. C., Michael, M. D., Winnay, J. N., Hayashi, T., Horsch, D., Accili, D., Goodyear, L. J., and Kahn, C. R. : A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Mol Cell* 2, 559-69 (1998)
55. Tamemoto, H., Kadowaki, T., Tobe, K., Yagi, T., Sakura, H., Hayakawa, T., Terauchi, Y., Ueki, K., Kaburagi, Y., Satoh, S., and *et al.* : Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1 [see comments]. *Nature* 372, 182-6 (1994)
56. Araki, E., Lipes, M. A., Patti, M. E., Bruning, J. C., Haag, B., 3rd, Johnson, R. S., and Kahn, C. R. : Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene [see comments]. *Nature* 372, 186-90 (1994)
57. Withers, D. J., Gutierrez, J. S., Towery, H., Burks, D. J., Ren, J. M., Previs, S., Zhang, Y., Bernal, D., Pons, S., Shulman, G. I., Bonner-Weir, S., and White, M. F. : Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 391, 900-4 (1998)
58. Bruning, J. C., Winnay, J., Bonner-Weir, S., Taylor, S. I., Accili, D., and Kahn, C. R. : Development of a novel polygenic model of NIDDM in mice heterozygous for IR and IRS-1 null alleles. *Cell* 88, 561-72 (1997)
59. Terauchi, Y., Tsuji, Y., Satoh, S., Minoura, H., Murakami, K., Okuno, A., Inukai, K., Asano, T., Kaburagi, Y., Ueki, K., Nakajima, H., Hanafusa, T., Matsuzawa, Y., Sekihara, H., Yin, Y., Barrett, J. C., Oda, H., Ishikawa, T., Akanuma, Y., Komuro, I., Suzuki, M., Yamamura, K., Kodama, T., Suzuki, H., Kadowaki, T., and *et al.* : Increased insulin sensitivity and hypoglycaemia in mice lacking the p85 alpha subunit of phosphoinositide 3-kinase. *Nat Genet* 21, 230-5 (1999)
60. Guillam, M. T., Hummler, E., Schaerer, E., Yeh, J. I., Birnbaum, M. J., Beermann, F., Schmidt, A., Deriaz, N., Thorens, B., and Wu, J. Y. : Early diabetes and abnormal postnatal pancreatic islet development in mice lacking Glut-2 [see comments] [published errata appear in *Nat Genet* 1997 Dec;17(4):503 and 1998 Jan;18(1):88]. *Nat Genet* 17, 327-30 (1997)
61. Katz, E. B., Stenbit, A. E., Hatton, K., DePinho, R., and Charron, M. J. : Cardiac and adipose tissue abnormalities but not diabetes in mice deficient in GLUT4 [see comments]. *Nature* 377, 151-5 (1995)
62. Grupe, A., Hultgren, B., Ryan, A., Ma, Y. H., Bauer, M., and Stewart, T. A. : Transgenic knockouts reveal a critical requirement for pancreatic beta cell glucokinase in maintaining glucose homeostasis. *Cell* 83, 69-78 (1995)
63. Terauchi, Y., Sakura, H., Yasuda, K., Iwamoto, K., Takahashi, N., Ito, K., Kasai, H., Suzuki, H., Ueda, O., Kamada, N., and *et al.* : Pancreatic beta-cell-specific targeted disruption of glucokinase gene. Diabetes mellitus due to defective insulin secretion to glucose. *J Biol Chem* 270, 30253-6 (1995)
64. Eriksson, U., and Swenne, I. : Diabetes in pregnancy: growth of the fetal pancreatic B cells in the rat. *Biol Neonate* 42, 239-48 (1982)
65. Swenne, I., and Eriksson, U. : Diabetes in pregnancy: islet cell proliferation in the fetal rat pancreas. *Diabetologia* 23, 525-8 (1982)
66. Hakan Borg, L. A., and Andersson, A. : Long-term effects of glibenclamide on the insulin production, oxidative metabolism and quantitative ultrastructure of mouse pancreatic islets maintained in tissue culture at different glucose concentrations. *Acta Diabetol Lat* 18, 65-83 (1981)
67. Hellerstrom, C. : The life story of the pancreatic B cell. *Diabetologia* 26, 393-400 (1984)

68. Finegood, D. T., Scaglia, L., and Bonner-Weir, S. : Dynamics of beta-cell mass in the growing rat pancreas. Estimation with a simple mathematical model. *Diabetes* 44, 249-56 (1995)
69. Brelje, T. C., Parsons, J. A., and Sorenson, R. L. : Regulation of islet beta-cell proliferation by prolactin in rat islets. *Diabetes* 43, 263-73 (1994)
70. Slack, J. M. : Developmental biology of the pancreas. *Development* 121, 1569-80 (1995)
71. Fujii, S. : Development of pancreatic endocrine cells in the rat fetus. *Arch Histol Jpn* 42, 467-79 (1979)
72. McEvoy, R. C. : Changes in the volumes of the A-, B-, and D-cell populations in the pancreatic islets during the postnatal development of the rat. *Diabetes* 30, 813-7 (1981)
73. Clark, A., and Grant, A. M. : Quantitative morphology of endocrine cells in human fetal pancreas. *Diabetologia* 25, 31-5 (1983)
74. Stefan, Y., Grasso, S., Perrelet, A., and Orci, L. : A quantitative immunofluorescent study of the endocrine cell populations in the developing human pancreas. *Diabetes* 32, 293-301 (1983)
75. Hellerstrom, C., and Swenne, I. : Growth pattern of pancreatic islets in animals. In The Diabetic Pancreas, Volk BW, and Arquilla ER, Eds. Plenum Press, New York, pp. 53-79 (1985)
76. Swenne, I. : The role of glucose in the *in vitro* regulation of cell cycle kinetics and proliferation of fetal pancreatic B-cells. *Diabetes* 31, 754-60 (1982)
77. Swenne, I. : Effects of aging on the regenerative capacity of the pancreatic B-cell of the rat. *Diabetes* 32, 14-9 (1983)
78. Rabinovitch, A., Quigley, C., Russell, T., Patel, Y., and Mintz, D. H. : Insulin and multiplication stimulating activity (an insulin-like growth factor) stimulate islet (beta-cell replication in neonatal rat pancreatic monolayer cultures. *Diabetes* 31, 160-4 (1982)
79. Hill, D. J., Petrik, J., and Arany, E. : Growth factors and the regulation of fetal growth. *Diabetes Care* 21 Suppl 2, B60-9 (1998)
80. Nielsen, J. H. : Effects of growth hormone, prolactin, and placental lactogen on insulin content and release, and deoxyribonucleic acid synthesis in cultured pancreatic islets. *Endocrinology* 110, 600-6 (1982)
81. Brelje, T. C., Allaire, P., Hegre, O., and Sorenson, R. L. : Effect of prolactin versus growth hormone on islet function and the importance of using homologous mammosomatotropic hormones. *Endocrinology* 125, 2392-9 (1989)
82. Brelje, T. C., and Sorenson, R. L. : Role of prolactin versus growth hormone on islet B-cell proliferation *in vitro*: implications for pregnancy. *Endocrinology* 128, 45-57 (1991)
83. Swenne, I., Hill, D. J., Strain, A. J., and Milner, R. D. : Growth hormone regulation of somatomedin C/insulin-like growth factor I production and DNA replication in fetal rat islets in tissue culture. *Diabetes* 36, 288-94 (1987)
84. Swenne, I., Heldin, C. H., Hill, D. J., and Hellerstrom, C. : Effects of platelet-derived growth factor and somatomedin-C/insulin- like growth factor I on the deoxyribonucleic acid replication of fetal rat islets of Langerhans in tissue culture. *Endocrinology* 122, 214-8 (1988)
85. Swenne, I., and Hill, D. J. : Growth hormone regulation of DNA replication, but not insulin production, is partly mediated by somatomedin-C/insulin-like growth factor I in isolated pancreatic islets from adult rats. *Diabetologia* 32, 191-7 (1989)
86. Van Schravendijk, C. F., Foriers, A., Van den Brande, J. L., and Pipeleers, D. G. : Evidence for the presence of type I insulin-like growth factor receptors on rat pancreatic A and B cells. *Endocrinology* 121, 1784-8 (1987)
87. Romanus, J. A., Rabinovitch, A., and Rechler, M. M. : Neonatal rat islet cell cultures synthesize insulin-like growth factor I. *Diabetes* 34, 696-702 (1985)
88. Swenne, I., Bone, A. J., Howell, S. L., and Hellerstrom, C. : Effects of glucose and amino acids on the biosynthesis of DNA and insulin in fetal rat islets maintained in tissue culture. *Diabetes* 29, 686-92 (1980)
89. Sjoholm, A., Welsh, N., and Hellerstrom, C. : Lithium increases DNA replication, polyamine content, and insulin secretion by rat pancreatic beta-cells. *Am J Physiol* 262, C391-5 (1992)
90. Sjoholm, A. : Phorbol ester stimulation of pancreatic beta-cell replication, polyamine content and insulin secretion. *FEBS Lett* 294, 257-60 (1991)
91. Sandler, S., and Andersson, A. : Long-term effects of exposure of pancreatic islets to nicotinamide *in vitro* on DNA synthesis, metabolism and B-cell function. *Diabetologia* 29, 199-202 (1986)
92. Dunger, A., Sjoholm, A., and Eizirik, D. L. : Amino acids and human amniotic fluid increase DNA biosynthesis in pancreatic islets of adult mouse, but this effect is lost following exposure to streptozotocin. *Pancreas* 5, 639-46 (1990)
93. Sjoholm, A., and Hellerstrom, C. : TGF-beta stimulates insulin secretion and blocks mitogenic response of pancreatic beta-cells to glucose. *Am J Physiol* 260, C1046-51 (1991)
94. Southern, C., Schulster, D., and Green, I. C. : Inhibition of insulin secretion from rat islets of Langerhans by interleukin-6. An effect distinct from that of interleukin-1. *Biochem J* 272, 243-5 (1990)
95. Sandler, S., Eizirik, D. L., Svensson, C., Strandell, E., Welsh, M., and Welsh, N. : Biochemical and molecular actions of interleukin-1 on pancreatic beta- cells. *Autoimmunity* 10, 241-53 (1991)
96. Sjoholm, A., Funakoshi, A., Efendic, S., Ostenson, C. G., and Hellerstrom, C. : Long term inhibitory effects of pancreastatin and diazepam binding inhibitor on pancreatic beta-cell deoxyribonucleic acid replication, polyamine content, and insulin secretion. *Endocrinology* 128, 3277-82 (1991)
97. Chen, Z. W., Agerberth, B., Gell, K., Andersson, M., Mutt, V., Ostenson, C. G., Efendic, S., Barros-Soderling, J., Persson, B., and Jornvall, H. : Isolation and characterization of porcine diazepam-binding inhibitor, a polypeptide not only of cerebral occurrence but also common in intestinal tissues and with effects on regulation of insulin release. *Eur J Biochem* 174, 239-45 (1988)
98. Tatemoto, K., Efendic, S., Mutt, V., Makk, G., Feistner, G. J., and Barchas, J. D. : Pancreastatin, a novel pancreatic peptide that inhibits insulin secretion. *Nature* 324, 476-8 (1986)
99. Efendic, S., Tatemoto, K., Mutt, V., Quan, C., Chang, D., and Ostenson, C. G. : Pancreastatin and islet hormone release. *Proc Natl Acad Sci U S A* 84, 7257-60 (1987)

100. Korcakova, L. : Mitotic division and its significance for regeneration of granulated B- cells in the islets of Langerhans in alloxan-diabetic rats. *Folia Morphol* 19, 24-30 (1971)
101. Steiner, H., Oelz, O., Zahnd, G., and Foresch, E. R. : Studies on islet cell regeneration, hyperplasia and intrainsular cellular interrelations in long lasting streptozotocin diabetes in rats. *Diabetologia* 6, 558-64 (1970)
102. McEvoy, R. C., and Hegre, O. D. : Morphometric quantitation of the pancreatic insulin-, glucagon-, and somatostatin-positive cell populations in normal and alloxan-diabetic rats. *Diabetes* 26, 1140-6 (1977)
103. Portha, B., Levacher, C., Picon, L., and Rosselin, G. : Diabetogenic effect of streptozotocin in the rat during the perinatal period. *Diabetes* 23, 889-95 (1974)
104. Bonner-Weir, S., Trent, D. F., Honey, R. N., and Weir, G. C. : Responses of neonatal rat islets to streptozotocin: limited B-cell regeneration and hyperglycemia. *Diabetes* 30, 64-9 (1981)
105. Dutrillaux, M. C., Portha, B., Roze, C., and Hollande, E. : Ultrastructural study of pancreatic B cell regeneration in newborn rats after destruction by streptozotocin. *Virchows Arch B Cell Pathol Incl Mol Pathol* 39, 173-85 (1982)
106. Leahy, J. L., Bonner-Weir, S., and Weir, G. C. : Minimal chronic hyperglycemia is a critical determinant of impaired insulin secretion after an incomplete pancreatectomy. *J Clin Invest* 81, 1407-14 (1988)
107. Lohr, M., Lubbersmeyer, J., Otremba, B., Klapdor, R., Grossner, D., and Kloppel, G. : Increase in B-cells in the pancreatic remnant after partial pancreatectomy in pigs. An immunocytochemical and functional study. *Virchows Arch B Cell Pathol Incl Mol Pathol* 56, 277-86 (1989)
108. Bonner-Weir, S., Trent, D. F., and Weir, G. C. : Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. *J Clin Invest* 71, 1544-53 (1983)
109. Clark, A., Bown, E., King, T., Vanhegan, R. I., and Turner, R. C. : Islet changes induced by hyperglycemia in rats. Effect of insulin or chlorpropamide therapy. *Diabetes* 31, 319-25 (1982)
110. Bonner-Weir, S., Deery, D., Leahy, J. L., and Weir, G. C. : Compensatory growth of pancreatic beta-cells in adult rats after short- term glucose infusion. *Diabetes* 38, 49-53 (1989)
111. Coleman, D. L. : Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. *Diabetologia* 14, 141-8 (1978)
112. Swenne, I., and Andersson, A. : Effect of genetic background on the capacity for islet cell replication in mice. *Diabetologia* 27, 464-7 (1984)
113. Westermark, P., and Wilander, E. : The influence of amyloid deposits on the islet volume in maturity onset diabetes mellitus. *Diabetologia* 15, 417-21 (1978)
114. Mares, J., and Welsh, M. : Expression of certain antiproliferative and growth-related genes in isolated mouse pancreatic islets: analysis by polymerase chain reaction. *Diabete Metab* 19, 315-20 (1993)
115. Sherr, C. J. : Cancer cell cycles. *Science* 274, 1672-7 (1996)
116. Grana, X., and Reddy, E. P. : Cell cycle control in mammalian cells: role of cyclins, cyclin dependent kinases (CDKs), growth suppressor genes and cyclin-dependent kinase inhibitors (CKIs). *Oncogene* 11, 211-9 (1995)
117. Hunter, T. : Oncoprotein networks. *Cell* 88, 333-46 (1997)
118. Dyson, N. : The regulation of E2F by pRB-family proteins. *Genes Dev* 12, 2245-62 (1998)
119. Mulligan, G., and Jacks, T. : The retinoblastoma gene family: cousins with overlapping interests. *Trends Genet* 14, 223-9 (1998)
120. Nigg, E. A. : Targets of cyclin-dependent protein kinases. *Curr Opin Cell Biol* 5, 187-93 (1993)
121. Morgan, D. O. : Cyclin-dependent kinases: engines, clocks, and microprocessors. *Annu Rev Cell Dev Biol* 13, 261-91 (1997)
122. LaBaer, J., Garrett, M. D., Stevenson, L. F., Slingerland, J. M., Sandhu, C., Chou, H. S., Fattaey, A., and Harlow, E. : New functional activities for the p21 family of CDK inhibitors. *Genes Dev* 11, 847-62 (1997)
123. Cheng, M., Olivier, P., Diehl, J. A., Fero, M., Roussel, M. F., Roberts, J. M., and Sherr, C. J. : The p21(Cip1) and p27(Kip1) CDK 'inhibitors' are essential activators of cyclin D-dependent kinases in murine fibroblasts. *Embo J* 18, 1571-83 (1999)
124. Sherr, C. J., and Roberts, J. M. : CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev* 13, 1501-12 (1999)
125. Reynisdottir, I., Polyak, K., Iavarone, A., and Massague, J. : Kip/Cip and Ink4 Cdk inhibitors cooperate to induce cell cycle arrest in response to TGF-beta. *Genes Dev* 9, 1831-45 (1995)
126. Reynisdottir, I., and Massague, J. : The subcellular locations of p15(Ink4b) and p27(Kip1) coordinate their inhibitory interactions with cdk4 and cdk2. *Genes Dev* 11, 492-503 (1997)
127. Alcorta, D. A., Xiong, Y., Phelps, D., Hannon, G., Beach, D., and Barrett, J. C. : Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. *Proc Natl Acad Sci U S A* 93, 13742-7 (1996)
128. Hara, E., Smith, R., Parry, D., Tahara, H., Stone, S., and Peters, G. : Regulation of p16CDKN2 expression and its implications for cell immortalization and senescence. *Mol Cell Biol* 16, 859-67 (1996)
129. Palmero, I., McConnell, B., Parry, D., Brookes, S., Hara, E., Bates, S., Jat, P., and Peters, G. : Accumulation of p16INK4a in mouse fibroblasts as a function of replicative senescence and not of retinoblastoma gene status. *Oncogene* 15, 495-503 (1997)
130. Serrano, M., Lin, A. W., McCurrach, M. E., Beach, D., and Lowe, S. W. : Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* 88, 593-602 (1997)
131. Zindy, F., Quelle, D. E., Roussel, M. F., and Sherr, C. J. : Expression of the p16INK4a tumor suppressor versus other INK4 family members during mouse development and aging. *Oncogene* 15, 203-11 (1997)
132. Morse, L., Chen, D., Franklin, D., Xiong, Y., and Chen-Kiang, S. : Induction of cell cycle arrest and B cell terminal differentiation by CDK inhibitor p18(INK4c) and IL-6. *Immunity* 6, 47-56 (1997)
133. Zindy, F., Soares, H., Herzog, K. H., Morgan, J., Sherr, C. J., and Roussel, M. F. : Expression of INK4 inhibitors of cyclin D-dependent kinases during mouse brain development. *Cell Growth Differ* 8, 1139-50 (1997)

134. Phelps, D. E., Hsiao, K. M., Li, Y., Hu, N., Franklin, D. S., Westphal, E., Lee, E. Y., and Xiong, Y. : Coupled transcriptional and translational control of cyclin-dependent kinase inhibitor p18INK4c expression during myogenesis. *Mol Cell Biol* 18, 2334-43 (1998)
135. Harbour, J. W., Luo, R. X., Dei Santi, A., Postigo, A. A., and Dean, D. C.: Cdk phosphorylation triggers sequential intramolecular interactions that progressively block Rb functions as cells move through G1. *Cell* 98, 859-69 (1999)
136. Swenne, I. : Islet cell thymidine kinase activity as indicator of islet cell proliferation in rat pancreas. *Diabetes* 39, 70-5 (1990)
137. Hanahan, D. : Heritable formation of pancreatic beta-cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes. *Nature* 315, 115-22 (1985)
138. Teitelman, G., Alpert, S., and Hanahan, D. : Proliferation, senescence, and neoplastic progression of beta cells in hyperplasic pancreatic islets. *Cell* 52, 97-105 (1988)
139. Marshall, C. J. : Tumor suppressor genes. *Cell* 64, 313-26 (1991)
140. Efrat, S., Baekkeskov, S., Lane, D., and Hanahan, D. : Coordinate expression of the endogenous p53 gene in beta cells of transgenic mice expressing hybrid insulin-SV40 T antigen genes. *Embo J* 6, 2699-704 (1987)
141. Welsh, M., Welsh, N., Nilsson, T., Arkhammar, P., Pepinsky, R. B., Steiner, D. F., and Berggren, P. O. : Stimulation of pancreatic islet beta-cell replication by oncogenes. *Proc Natl Acad Sci U S A* 85, 116-20 (1988)
142. Rane, S. G., Dubus, P., Mettus, R. V., Galbreath, E. J., Boden, G., Reddy, E. P., and Barbacid, M. : Loss of Cdk4 expression causes insulin-deficient diabetes and Cdk4 activation results in beta-islet cell hyperplasia. *Nat Genet* 22, 44-52 (1999)
143. Sicinski, P., Donaher, J. L., Parker, S. B., Li, T., Fazeli, A., Gardner, H., Haslam, S. Z., Bronson, R. T., Elledge, S. J., and Weinberg, R. A. : Cyclin D1 provides a link between development and oncogenesis in the retina and breast. *Cell* 82, 621-30 (1995)
144. Sicinski, P., Donaher, J. L., Geng, Y., Parker, S. B., Gardner, H., Park, M. Y., Robker, R. L., Richards, J. S., McGinnis, L. K., Biggers, J. D., Eppig, J. J., Bronson, R. T., Elledge, S. J., and Weinberg, R. A. : Cyclin D2 is an FSH-responsive gene involved in gonadal cell proliferation and oncogenesis. *Nature* 384, 470-4 (1996)
145. Geng, Y., Whoriskey, W., Park, M. Y., Bronson, R. T., Medema, R. H., Li, T., Weinberg, R. A., and Sicinski, P. : Rescue of cyclin D1 deficiency by knockin cyclin E. *Cell* 97, 767-77 (1999)
146. Zuo, L., Weger, J., Yang, Q., Goldstein, A. M., Tucker, M. A., Walker, G. J., Hayward, N., and Dracopoli, N. C. : Germline mutations in the p16INK4a binding domain of CDK4 in familial melanoma. *Nat Genet* 12, 97-9 (1996
147. Wolfel, T., Hauer, M., Schneider, J., Serrano, M., Wolfel, C., Klehmann-Hieb, E., De Plaen, E., Hankeln, T., Meyer zum Buschenfelde, K. H., and Beach, D. : A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. *Science* 269, 1281-4 (1995)
148. Lacy, P. E., and Kostianovsky, M. : Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* 16, 35-9 (1967)
149. Hellerstrom, C. H., Lewis, N. J., Borg, H., Johnson, R., and Freinkel, N. : Method for large-scale isolation of pancreatic islets by tissue culture of fetal rat pancreas. *Diabetes* 28, 769-76 (1979)
150. Weir, G. C., and Bonner-Weir, S. : Islet transplantation as a treatment for diabetes. *J Am Optom Assoc* 69, 727-32 (1998)

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