Lessons from neonatal β-cell epigenomic for diabetes prevention and treatment

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Abstract

Pancreatic β-cell expansion and functional maturation during the birth-to-weaning period plays an essential role in the adaptation of plasma insulin levels to metabolic needs. These events are driven by epigenetic programs triggered by growth factors, hormones and nutrients. These mechanisms operating in the neonatal period can be at least in part reactivated in adult life to increase the functional β-cell mass and face conditions of increased insulin demand such as obesity or pregnancy. In this review, we will highlight the importance of studying these signalling pathways and epigenetic programs to understand the causes of different forms of diabetes and to permit the design of novel therapeutic strategies to prevent and treat this metabolic disorder affecting hundreds of million people worldwide.

β-cell replication, newborn, epigenetic, weaning, obesity, diabetes

Highlights

Expansion of β-cell mass during suckling period relies on epigenetic program connected to specific signalling pathways of humoral factors

Maternal milk, innate immunity and intestinal microbiota are keys for the control of the epigenetic mechanisms governing cell cycle entry pathways during the lactating period

Nutritional switch during suckling-weaning transition induces a change in the epigenetic pathways leading to the growth arrest and β-cell maturation

Pathways involved in postnatal constitutive and adaptive β-cell replication are reactivated during pregnancy and obesity

In diabetes, activation of epigenetic pathways involved in neonate β-cell proliferation is impaired

Understanding how to manipulate these pathways can be foreseen as a possible therapeutic treatment option to restore the lost BCM in diabetes.

Insulin, secreted by pancreatic β-cells residing within the islet of Langerhans, is the only hypoglycemic hormone promoting glucose utilization and storage in insulin-sensitive tissues. The amount of insulin released is precisely adjusted to meet the organism needs and avoid chronic hyperglycemia or life-threatening hypoglycemic episodes. This is achieved, thanks to a continuous adaptation of the mass and secretory activity of β-cells. This adaptive capacity has inspired academics, clinicians and pharmaceutical companies in search for novel strategies to prevent and treat diabetes mellitus, a chronic metabolic disorder expected to affect more than 600 million people by 2040. In mammals, β-cells continue to expand during the birth-to-weaning period before achieving full secretory competence [1]. These early postnatal events are characterised by strong epigenetic plasticity [2], permitting to couple changes in the nutritional environment to reprogramming of gene expression and of cellular activities. The study of the mechanisms underlying these phenomena, provides a unique opportunity to identify humoral factors and intracellular pathways driving β-cell proliferation and the acquisition of full secretory competence. This review provides an overview of the key signalling pathways connecting changes in nutrients, growth factors and hormones occurring during the suckling and weaning period to epigenetic mechanisms that control β-cell mass expansion and functional maturation. A better understanding of these mechanisms will be instrumental for the generation of fully operational insulin-secreting cells capable of replacing β-cells that are lost in Type 1 diabetes (T1D) patients. These pathways functioning during the neonatal period can be reactivated later in life to compensate for weight gain, pregnancy or a decrease in insulin sensitivity. Thus, a detailed knowledge of the components of these signalling cascades may also pave the way to novel therapeutic strategies to treat Type 2 (T2D) and gestational diabetes.

β-cells during the suckling period: functionally immature but optimised for proliferation

Since the proliferative capacity of β-cells in adults is limited, expansion of insulin-secreting cells during the suckling period is critical to generate enough cells to control blood glucose levels throughout life. In rodents, the expansion of the BCM results from increased β-cell size and replication [3,4], but also from duct cell differentiation that accounts for 30%–50% of the insulin-secreting cells produced during the first postnatal month [5,6]. In human, the BCM is largely established before the age of 20 [7] and the expansion, which mostly relies on β-cell replication [3], occurs mainly during the first 5 years [8,9]. The mechanisms involved in cell cycle regulation include the interconnection of kinase cascades (e.g. Jak2/Stat5, Nfat, mTorc1, Mapk/Jnk3) with transcription factors and non-coding RNAs such as microRNAs and long non-coding RNAs (**Box 1**). During the suckling period, cell cycle entry is triggered by

different cyclins [10,11]. These mechanisms are not specific to β-cells, suggesting that neonate β-cells are equipped with specific receptors and/or transporters that connect signals such as nutrients and/or growth factors (GFs) to classical proliferative pathways. A major source of these humoral factors is the maternal milk. The importance of milk quality for neonatal β-cell growth has been confirmed by exposing postpartum females to under- or overnutrition during the suckling period [13]. Indeed, this alters milk composition and can lead to premature perinatal maturation in the offspring [13]. Likewise, in rodents maternal under- or overnutrition during lactation is accompanied by alteration of neonate β-cell proliferation, resulting in a reduced BCM [14–16]. Maternal milk contains vitamins, immune cells, hormones, prebiotics, microbiota and various GFs [17]. Newborn β-cells express several GF and hormone receptors capable of inducing Ccnd1/2 expression through activation of signalling pathways (**Box 2**). The presence of these receptors is essential to allow GFs and hormones particularly abundant in the neonate islet microenvironment to modulate β-cell proliferation.

Changes in maternal nutrition during gestation can affect postnatal β-cell functions during the lactation period. In rodents, maternal caloric restriction (CR) or low- protein (LP) diet during gestation leads to reduced birthweight and to a reduction in the offspring's BCM at birth [18], reflecting an imbalance between β-cell replication and apoptosis [19]. Later in life, this can affect the compensatory capacity of β-cells under conditions of increased insulin needs, favouring diabetes development [19]. β-cell proliferation is also compromised in neonatal rats and mice born from dams fed with a high-fat diet during gestation [20,21]. Reduced BCM caused by maternal undernutrition or overnutrition may be linked to changes in the level of GFs in maternal milk [22]. However, the gut and the innate immune system could also be a source of GFs. Indeed, β-cell expansion and maturation coincide with the development of intestinal and innate immunity (II). During suckling, intestinal tract colonization by commensal microbes contributes to microbiota formation and intestinal barrier maturation [23]. Interestingly, in zebrafish, administration of gram-negative *Aeromonas bacterial species* in the intestine of germ-free larvae stimulates β-cell proliferation and BCM expansion [24]. It is not yet known whether intestinal bacteria have analogous effects in mammals. Gut microbiota maturation during suckling is accompanied by increased circulating levels of Glucagon-like peptide 1 (GLP-1) [25], an enteroendocrine hormone produced by intestinal L-cell. GLP-1 stimulates β-cell proliferation and neogenesis in adult rodents [26] and inhibits β-cell apoptosis [27]. Beside GLP-1, GFs released by II cells that are particularly abundant in neonate islets may also promote BCM expansion [28]. At birth, II cells develop to protect neonates against infections originating from the extrauterine environment [29] but regulate also tissue growth during

development and repair [30]. In newborn islets, $CCR2^+$ myeloid cells produce several mitogenic factors, including insulin-like growth factor 2, follistatin-like proteins and connective tissue growth factors [28]. The absence of $CCR2^+$ cells leads to a reduced BCM [28], suggesting that mitogenic factors secreted by islet-resident II cells play a key role in β-cell proliferation during the suckling period [28,31].

Epigenetic programming of β-cell proliferation and metabolism during the suckling period

Adaptation to maternal milk nutrition requires profound modifications in gene expression. This is achieved through reprogramming of the DNA and RNA methylation profile as well as in changes in histone methylation, acetylation, phosphorylation or ubiquitylation. These epigenetic mechanisms are crucial for activating the expression of genes controlling metabolism and proliferation of immature β-cell during the breastfeeding period [32,33]. In immature β-cells, the expression of genes involved in cell cycle control and in anaerobic glycolysis correlates with changes in histone 3 trimethylation at lysine 27 and 4 (H3K27me3 and H3K4me3, respectively) [34-36]. H3K4 trimethylation at gene promoters and distal regulatory enhancers favours the recruitment of the transcription machinery and of chromatin remodelling complexes [37] and is generally accompanied by H3K27 acetylation and low levels of the repressive H3K27me3 marks. On the contrary, gene repression occurs at sites in which H3K27me3 is abundant while H3K4me3 is rare [38]. In neonatal β-cells, the CDK inhibitor genes Cdkn1a and Cdkn1c display elevated levels of repressive H3K27me3 marks while the gene coding for c-Myc, which plays a key role in proliferation, shows an enrichment in activating H3K4me3 marks [39]. Likewise, H3K4me3 is abundant in the regulatory regions of the genes coding for hexokinase (Hk) 3 and lactate/proton symporter monocarboxylate transporter-1 (Mct1), two enzymes essential for β-cell insulin secretion in response to low glucose and pyruvate, respectively [39,40]. Trimethylation of H3K27 and H3K4 is regulated by histone methyltransferase (HMT) and demethylase (HDM). HMT and HDM activities are controlled by key signalling pathways during the suckling period. The enhancer of zeste homolog 2 (EZH2) is a HMT subunit of the polycomb repressive complex 2 (PRC2) that trimethylates H3K27 [41]. mTorc1 signalling positively regulates EZH2 activity [42,43], resulting in Cdkn1c downregulation and β-cell proliferation [42,43]. Other findings support a role for mTorc1 signaling in the epigenetic regulation of immature β-cell functions. Indeed, mTorc1 diminishes the expression of DNA methyl transferase 3a (Dnmt3a), a critical *de novo*

DNA epigenetic modifier [44]. This leads to hypomethylation and, thereby, activation of genes coding for Hk1, Hk2 and lactate dehydrogenase (Ldha) [44]. The same mechanism appears to apply to many genes involved in proliferation. Indeed, during the suckling period, 30% of the genes involved in G1/S phase progression and 59% of the genes involved in G2/M are upregulated by mTorc1 signaling [42]. Therefore, mTorc1 is likely to promote proliferation via global hypomethylation of cell cycle-related genes. Several GFs operating during the suckling period are able to trigger mTorc1 signaling, resulting in modifications in DNA and histone methylation [45,46]. Epigenetic regulation of gene expression in immature β-cells triggered by mTorc1 requires inhibition of the Smad pathway. In general, upon activation Smad protein complexes accumulate in the nucleus where they participate to the recruitment of HMT subunits, leading to H3K4 trimethylation, and induction of gene expression [47]. Several genes involved in proliferation, including the cell cycle blocker Cdkn2a, are regulated by Smads possibly through H3K4 trimethylation [48]. During the suckling period, inactivation of the Smad pathway leads to a reduction of H3K4me3 and to Cdkn2a silencing and repression of genes necessary for β-cell maturation [49]. Inappropriate activation of Smads during this critical period, results in the induction of Cdkn2a, which in turn, stops neonatal β-cell proliferation and in premature maturation [48]. Fetuin-A, a glycoprotein released by the liver, inhibits the Smad pathway in neonatal islets [48], supporting the idea that humoral factors in maternal milk and/or released by developing organs orchestrate the epigenetic programming of immature β-cells during the suckling period.

The nutritional switch during the suckling-weaning transition promotes β-cell maturation

Full β-cell maturation is achieved upon weaning [50], when infants switch from maternal breastmilk feeding, rich in fat and poor in carbohydrates, to solid and carbohydrate-enriched food. This is associated with changes in the gastrointestinal tract enabling the digestion and absorption of solid foods. During weaning, β-cells acquire the ability to sense changes in blood glucose levels and metabolize glucose to produce metabolic signals that trigger exocytosis of insulin-containing granules. Recent studies suggest that weaning is also crucial to generate β-cell subpopulations with different insulin secretion kinetics and amplitudes [51]. The functional heterogeneity of β-cells could play a central role in the fine-tuning of insulin secretion in response to variations of plasma glucose [52,53]. β-cell terminal differentiation is likely to be achieved in two sequential stages, including the repression of the immature phenotype and the appearance of glucose-responsiveness [44,54]. In this regard, research

carried out in the last few years suggests that the nutritional change drives major epigenetic reprogramming in β-cells (**Fig. 1**), permitting to stop proliferation and to reduce anaerobic glycolysis. Fetuin-A concentration declines from birth to weaning [48] while the islet concentration of TGFβ, a strong inducer of Smad pathway, increases [55], allowing H3K4 trimethylation of Cdkn2a, Cdkn1a and Cdkn1c, and causing cell cycle arrest [35,48]. Blockade of proliferation might also result from the decline in the expression of GFs receptors as exemplified by the HGF receptor c-Met. During weaning, the expression of MEG3 increases [41] and this lncRNA forms a complex with EZH2. This leads to a rise in H3K27 trimethylation in the c-Met promoter with a consequent reduction of β-cell proliferation [41]. The expression of PDGFRA is also reduced during weaning, in this case as a consequence of changes in the miRNA profile, in particular a rise of miR-29 [56]. This miRNA can profoundly affect also basal insulin secretion by silencing Mct-1 and the transcriptional repressor REST [7,57,58]. The genes coding for Ldha, Hk1 and Hk2, which are major players in the anaerobic glycolysis of neonatal β-cells, are also silenced during the suckling-weaning transition. During this period, DNA methylation of the genes coding for these enzymes increases due to enhanced Dnmt3a activity [44], which coincides with a switch from mTorc1 to AMPK and mTorc2 signalling [44,59]. Silencing of Ldha, Hk1 and Hk2 allows β-cells to switch glucose metabolism from anaerobic glycolysis towards oxidative phosphorylation. A drop in FA and a rise in blood glucose levels are probably major drivers of the modification in mTorc signalling. Indeed, silencing of mTorc1 and DNA methylation occur concomitantly when maternal milk is replaced in the diet by carbohydrate consumption [44, 57]. In mice, continuing milk fat consumption from the neonatal period until adulthood maintains mTorc1 signalling and β-cells remain immature [57]. Moreover, glucose promotes important changes in the DNA methylation profile of many genes [58]. In mice, increased expression of genes controlling glucose-induced insulin secretion including Znt8, NeuroD1, Glut2, Urocortin 3 (Ucn3) and Kir6.2 relies on H3K4 trimethylation [36]. Elevated transcription of Pdx1, Abcc8, Syt4/7 and Snap25 has been associated with chromatin opening due to H3K27me3. Gene activation during the second stage of β-cell maturation includes also histone H4 arginine 3 asymmetric dimethylation (H4R3me2a). This epigenetic mark is mediated by protein arginine methyltransferase 1 (Prmt1) [59]. In neonatal islets, the genes coding for the transcription factors Nkx6.1, Nkx2.2, MafA, NeuroD1 and Pdx1 are enriched with the H4R3me2a. The expression of these genes is further enhanced at weaning by RNA adenosine methylation mediated by methyltransferase-like 3 and 4 [54]. These transcription factors activate thousands of genes involved in glucose oxidative

phosphorylation and sensing, and induce the expression of ion channels subunits, mitochondrial shuttles, electron transport chain proteins and of components of the secretory machinery [59-61].

The epigenomic code of neonatal β-cells as a paradigm for adaptative proliferation under conditions of increased insulin demand

After weaning, the BCM remains relatively stable. However, under conditions of increased insulin demand an expansion of the BCM can be observed [62]. Obesity and pregnancy are associated with a diminished sensitivity of insulin target tissues. Thus, expansion of the BCM is pivotal for providing enough insulin and preserving normoglycemia [63,64]. BCM expansion in obesity and pregnancy necessitates a reduction in the expression of CDK inhibitors and a rise in the level of different cyclins, an observation confirmed in the islets from obese non-diabetic donors [65-68]. The re-expression of cell cycle-related genes is associated with DNA hypomethylation and ectopic expression of c-Myc [69]. In addition, the expression of ncRNAs is induced and proliferation pathways (e.g. JNK3, Nfatc2) are activated [68,70,71], suggesting that β-cell adaptation to an increased metabolic demand may rely on a subset of β-cells returning to an immaturity, neonatal-like, status via epigenetic reprogramming [57] (**Fig. 2, Key Figure**). At present, we don't know whether this reprogramming is always associated with a reduction in glucose responsiveness or if part of the cells may proliferate while retaining glucose-dependent insulin secretion.

In obesity and pregnancy, the metabolic demand comes from nutrient overload and systemic insulin resistance [72]. Release of FA from insulin-resistant adipocyte and the postprandial lipid fluctuations, results in an increased plasma FA concentration. This may create a FA-enriched environment within the islets, potentially mimicking the conditions occurring during the suckling period. The fact that FA modify H3K27 trimethylation [73], and induce a global change in DNA methylation in human islets [74] supports the hypothesis of a role of lipids in β-cell adaptation to obesity and pregnancy. In obesity and pregnancy there is also an increase in local and circulating GF, including HGF, IGF-1, PDGF and mitogenic hormones such as prolactin [46,75,76]. Beside GF and lipids, cytokines may also play a role in these adaptive mechanisms as suggested by the presence of T-cells infiltrating the islets very early in the development of diabetes, already in the stage of glucose intolerance [77]. This environment may reactivate the epigenetic profile of immature β-cells, causing the cells to resume proliferation. This immature and proliferative state induced by metabolic overload is probably

transient and under normal conditions most β-cells undergoing this process will recover full glucose responsiveness. Failure of proliferative β-cells to recover full secretory competence may contribute to the loss of functional BCM in type 2 diabetes (T2D). Islets from patients with T2D have been shown to harbor also some dedifferentiated β-cells displaying progenitor-like characteristics [78].

Unlike immature neonatal β-cells that proliferate during the suckling period, dedifferentiated cells do not produce insulin and express several markers of progenitor cells. If these dedifferentiated cells do or can proliferate remain un unmet question. Nonetheless, it is possible that these dedifferentiated cells coexist with proliferating immature β-cells. Indeed, neonatal-like β-cells have been identified in islets of T2D patients and in mice model of T2D [57,69]. These cells express insulin but are poorly responsive to glucose. They are characterized by mTorc1 activation, elevation of c-Myc expression and broad hypomethylation of cell cycle genes. In T2D, genetic predisposition with persistent metabolic overload could lead to alterations in epigenetic reprogramming. Conversely, epigenetic reprograming by metabolic overload could also influence predisposing genes either affecting the capacity of β-cells to resume proliferation or causing dedifferentiation to a progenitor-like state. The latter is supported by Mendelian randomization (MR) analysis using GWAS single nucleotide polymorphisms (SNPs) linked to T1D and DNA methylation levels at non-HLA loci [79]. MR shows that DNA methylation levels causally influence T1D risk, and thereby, might lead to β-cell infiltration by T-cells [79]. Chronic hyperlipidemia and hyperglycemia induce major changes in DNA methylome and could be the drivers of this epigenomic dysregulation leading to different degrees of β-cells immaturity and loss of functional BCM [58,74].

Translating the knowledge of neonatal β-cell physiology into therapy.

Restoration of a functional BCM sufficient to compensate for insulin resistance is crucial for achieving long-term glycemic control in diabetes (**Box 3**). Thus, targeting the epigenetic mechanisms controlling β-cell replication and maturation is an attractive strategy to prevent and treat T2D. Some current medications that stimulate insulin secretion, including sulfonylurea and GLP-1 receptor agonists (GLP-1RA) promote changes in DNA methylation [80,81]. The modifications of the DNA methylome triggered by GLP-1RA are correlated with NAFTc1 activation, induction of cell cycle regulators and β-cell replication [82,83]. In addition, GLP-1RA stimulates mTorc1 [84] and Jnk activity [68]. Although GLP-1RA improves glycemic control, full normalization of blood glucose levels is not readily observed in patients

treated with any of the available GLP-1 mimetics [85]. In addition, like sulfonylurea, these drugs can have adverse secondary effects and are inefficient in preserving long-term glycemic control [85]. Chronic use of these compounds may even lead to deterioration of β-cell function [86]. These concerns prompted the search for alternative drugs with mitogenic properties and less side effects. In this perspective, GF involved in postnatal BCM expansion may be attractive candidates, as exemplified by HGF, which has been reported to induce Ccnd1 and Ccnd2 expression and promote β-cell proliferation under insulin resistance conditions [87]. In addition, HGF improves systemic insulin sensitivity and inflammation and does not lead to cancers in individuals without genetic abnormalities in the c-Met receptor [87]. However, synthetic HGF production costs are prohibitive and the manufactured protein is unstable, preventing its clinical application [88]. Alternative molecules mimicking the effect of HGF are now considered promising candidates for T2D treatment [88].

Beside inspiring the search for new therapeutic compounds, current knowledge of the processes occurring in the postnatal period suggest that preservation of neonate BCM expansion and maturation can prevent diabetes development in adulthood. This hypothesis is supported by two population-based studies showing that children who are never breastfed have a twofold increased risk of T1D when compared with those who were breastfed [89]. Management of breastfeeding duration and of quality of maternal milk, could help maintaining the epigenetic program of neonate β-cells and the level of nutrients and growth factors crucial for stimulating the mitogenic pathways. This is in line with the recommendations of the World Health Organisation which support exclusive breastfeeding for infants for the first six months of life, with nutritionally adequate foods, to continue up to two years of age or beyond [90].

Transplantation of insulin-secreting cells produced from human stem cells constitute an attractive approach for T1D treatment, which results from autoimmune destruction of β-cells. Islets or pancreas transplantation can be an alternative to insulin injections when appropriate glycemic control is not achieved by conventional treatment. However, this practice remains marginal as islets from two or three human donors are required for each recipient. To circumvent this issue, cell transplantation strategies based on alternative and unlimited sources of surrogate β-cells have been envisaged. At present, β-cells generated from stem cells are still not functionally equivalent to fully mature β–cells [91]. It is postulated that the signalling pathways driving cell replication remain active in β-cells derived from stem cells and represent a limiting factor for the differentiation of fully functional insulin-secreting cells [91]. Thus, targeting the epigenetic program that account for β-cell maturation using humoral factors

driving this stage during postnatal development of β-cells and/or pharmacological manipulation of epigenetic modifier may potentially improve the generation β-cell surrogates for transplantation purposes.

Concluding Remarks and Future Perspectives

Here, we highlighted the signals and epigenetic pathways governing early postnatal β-cell proliferation and maturation. These pathways can be at least in part reactivated in adulthood to allow the adaptation of β-cells to metabolic conditions of increased insulin demand. Several questions remain (see outstanding questions), including the connection between the mitogenic pathways and epigenetic mechanisms, and the impact of long-term reactivation of these pathways on insulin secretion. We believe that activation of these mechanisms allows β-cell compensation to metabolic cues and thereby, can be a promising strategy for restoring the functional BCM in diabetes. Finally, we propose that studying the humoral factors involved in postnatal BCM expansion can potentially lead to the identification of next natural-based antidiabetic.

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Conflict of interest statement

The authors declare no competing interests

References

1 Ferner, K. *et al.* (2017) Comparative anatomy of neonates of the three major mammalian groups (monotremes, marsupials, placentals) and implications for the ancestral mammalian neonate morphotype. *J. Anat.* 231, 798–822

2 Marousez, L. *et al.* (2019) Epigenetics: Linking Early Postnatal Nutrition to Obesity Programming? *Nutrients* 11, 2966

3 Anzi, S. *et al.* (2018) Postnatal Exocrine Pancreas Growth by Cellular Hypertrophy Correlates with a Shorter Lifespan in Mammals. *Dev. Cell* 45, 726-737.e3

4 Bader, E. *et al.* (2016) Identification of proliferative and mature β-cells in the islets of Langerhans. *Nature* 535, 430–434

5 Al-Hasani, K. *et al.* (2013) Adult Duct-Lining Cells Can Reprogram into β-like Cells Able to Counter Repeated Cycles of Toxin-Induced Diabetes. *Dev. Cell* 26, 86–100

6 El-Gohary, Y. *et al.* (2016) Intraislet Pancreatic Ducts Can Give Rise to Insulin-Positive Cells. *Endocrinology* 157, 166–175

7 Cnop, M. *et al.* (2011) Longevity of human islet α- and β-cells. *Diabetes Obes. Metab.* 13 Suppl 1, 39–46

8 Wang, P. *et al.* (2015) Diabetes mellitus--advances and challenges in human β-cell proliferation. *Nat. Rev. Endocrinol.* 11, 201–212

9 Meier, J.J. *et al.* (2008) Beta-cell replication is the primary mechanism subserving the postnatal expansion of beta-cell mass in humans. *Diabetes* 57, 1584–94

10 Rubin, S.M. (2013) Deciphering the retinoblastoma protein phosphorylation code. *Trends Biochem. Sci.* 38, 12–19

11 Kushner, J.A. *et al.* (2005) Cyclins D2 and D1 Are Essential for Postnatal Pancreatic β-Cell Growth. *Mol. Cell. Biol.* 25, 3752–3762

12 Chen, H. *et al.* (2015) Augmented Stat5 Signaling Bypasses Multiple Impediments to Lactogen-Mediated Proliferation in Human β-Cells. *Diabetes* 64, 3784–3797

13 Ellsworth, L. *et al.* (2018) Lactational programming of glucose homeostasis: A window of opportunity. *Reproduction* 156, R23–R42

14 Jacovetti, C. *et al.* (2015) Postnatal β-cell maturation is associated with islet-specific microRNA changes induced by nutrient shifts at weaning. *Nat. Commun.* 6, ncomms9084

15 Huang, Y.-H. *et al.* (2017) Maternal high-fat diet impairs glucose metabolism, β-cell function and proliferation in the second generation of offspring rats. *Nutr. Metab.* 14,

16 Su, Y. *et al.* Maternal low protein isocaloric diet suppresses pancreatic β-cell proliferation in mouse offspring via miR-15b. *Endocrinology*, 157. (2016) , 4782–4793

17 Christian, P. *et al.* (2021) The need to study human milk as a biological system. *Am. J. Clin. Nutr.* 113, 1063–1072

18 Boehmer, B.H. *et al.* (2017) The impact of IUGR on pancreatic islet development and β-cell function. *J. Endocrinol.* 235, R63–R76

19 King, R. *et al.* (2019) Offspring of Mice Exposed to a Low-Protein Diet in Utero Demonstrate Changes in mTOR Signaling in Pancreatic Islets of Langerhans, Associated with Altered Glucagon and Insulin Expression and a Lower β-Cell Mass. *Nutrients* 11, 605

20 Cerf, M.E. *et al.* (2005) Islet cell response in the neonatal rat after exposure to a high-fat diet during pregnancy. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* 288, R1122-R1128

21 Zheng, J. *et al.* (2020) Maternal high-fat diet regulates glucose metabolism and pancreatic β cell phenotype in mouse offspring at weaning. *PeerJ* 8, e9407

22 Munblit, D. *et al.* (2018) Levels of Growth Factors and IgA in the Colostrum of Women from Burundi and Italy. *Nutrients* 10, 1216

23 Beaumont, M. *et al.* (2020) Gut microbiota derived metabolites contribute to intestinal barrier maturation at the suckling-to-weaning transition. *Gut Microbes* 11, 1268–1286
24 Hill, J.H. *et al.* (2016) A conserved bacterial protein induces pancreatic beta cell expansion during zebrafish development. *eLife* 5, 18
25 Padidela, R. *et al.* (2009) Elevated basal and post-feed glucagon-like peptide 1 (GLP-1) concentrations in the neonatal period. *Eur. J. Endocrinol.* 160, 53–58
26 Chon, S. and Gautier, J.F. (2016) An update on the effect of incretin-based therapies on β-cell function and mass. *Diabetes and Metabolism Journal* 40, 99–114
27 Drucker, D.J. (2006) The biology of incretin hormones. *Cell Metab.* 3, 153–165
28 Mussar, K. *et al.* (2017) A CCR2+ myeloid cell niche required for pancreatic β cell growth. *JCI Insight* 2, e93834
29 Yu, J.C. *et al.* (2018) Innate Immunity of Neonates and Infants. *Front. Immunol.* 9, 1759
30 Pollard, J.W. (2009) Trophic macrophages in development and disease. *Nat. Rev. Immunol.* 9, 259–270
31 Van Gassen, N. *et al.* (2015) Concise Review: Macrophages: Versatile Gatekeepers During Pancreatic β-Cell Development, Injury, and Regeneration. *STEM CELLS Transl. Med.* 4, 555–563
32 Butruille, L. *et al.* (2019) Maternal high-fat diet during suckling programs visceral adiposity and epigenetic regulation of adipose tissue stearoyl-CoA desaturase-1 in offspring. *Int. J. Obes.* 43, 2381–2393
33 Kawahori, K. *et al.* (2020) Ascorbic acid during the suckling period is required for proper DNA demethylation in the liver. *Sci. Rep.* 10, 21228
34 Arda, H.E. *et al.* (2016) Age-Dependent Pancreatic Gene Regulation Reveals Mechanisms Governing Human β Cell Function. *Cell Metab.* 23, 909–920
35 Wang, P. *et al.* (2019) Combined Inhibition of DYRK1A, SMAD and Trithorax Pathways Synergizes to Induce Robust Replication in Adult Human Beta Cells. *Cell Metab.* 29, 638-652.e5
36 Campbell, S.A. *et al.* (2021) H3K4 Trimethylation Is Required for Postnatal Pancreatic Endocrine Cell Functional Maturation. *Diabetes* 70, 2568–2579
37 Jiang, H. (2020) The complex activities of the SET1/MLL complex core subunits in development and disease. *Biochim. Biophys. Acta Gene Regul. Mech.* 1863, 194560
38 Wiles, E.T. and Selker, E.U. (2017) H3K27 methylation: a promiscuous repressive chromatin mark. *Curr. Opin. Genet. Dev.* 43, 31–37
39 Puri, S. *et al.* (2018) Replication confers β cell immaturity. *Nat. Commun.* 9, 485
40 Rutter, G.A. *et al.* (2020) Metabolic and functional specialisations of the pancreatic beta cell: gene disallowance, mitochondrial metabolism and intercellular connectivity. *Diabetologia* 63, 1990–1998
41 Iyer, S. *et al.* (2017) Long Noncoding RNA MEG3 Is an Epigenetic Determinant of Oncogenic Signaling in Functional Pancreatic Neuroendocrine Tumor Cells. *Mol. Cell. Biol.* 37, e00278-17
42 Wang, Y. *et al.* (2019) Dual Effect of Raptor on Neonatal β-Cell Proliferation and Identity Maintenance. *Diabetes* 68, 1950–1964
43 Ni, Q. *et al.* (2017) Raptor regulates functional maturation of murine beta cells. *Nat. Commun.* 8, 15755
44 Dhawan, S. *et al.* (2015) DNA methylation directs functional maturation of pancreatic β cells. *J. Clin. Invest.* 125, 2851–2860
45 Maity, S. *et al.* (2020) TGFβ acts through PDGFRβ to activate mTORC1 via the Akt/PRAS40 axis and causes glomerular mesangial cell hypertrophy and matrix protein expression. *J. Biol. Chem.* 295, 14262–14278

46 Abderrahmani, A. *et al.* (2018) Increased Hepatic PDGF-AA Signaling Mediates Liver Insulin Resistance in Obesity-Associated Type 2 Diabetes. *Diabetes* 67, 1310–1321
47 Bertero, A. *et al.* (2015) Activin/nodal signaling and NANOG orchestrate human embryonic stem cell fate decisions by controlling the H3K4me3 chromatin mark. *Genes Dev.* 29, 702–717
48 Gerst, F. *et al.* (2021) The hepatokine fetuin-A disrupts functional maturation of pancreatic beta cells. *Diabetologia* 64, 1358–1374
49 Richardson, C.C. *et al.* (2015) Increased perinatal remodelling of the pancreas in somatostatin-deficient mice: potential role of transforming growth factor-beta signalling in regulating beta cell growth in early life. *Horm. Metab. Res. Horm. Stoffwechselforschung Horm. Metab.* 47, 56–63
50 Liu, J.S.E. and Hebrok, M. (2017) All mixed up: defining roles for β-cell subtypes in mature islets. *Genes Dev.* 31, 228–240
51. Dorrell, C. *et al.* (2016) Human islets contain four distinct subtypes of β cells. *Nat. Commun.* 7, 11756
52. Da Silva, X. and Rutter, G.A. (2020) Metabolic and Functional Heterogeneity in Pancreatic β Cells. *J. Mol. Biol* 5,1395-1406.
53. Pipeleers, D. *et al*. (2017) Heterogeneity in the Beta-Cell Population: a Guided Search Into Its Significance in Pancreas and in Implants. *Curr. Diab. Re.* 17, 86
54 Wang, Y. *et al.* (2020) m6A mRNA Methylation Controls Functional Maturation in Neonatal Murine β-Cells. *Diabetes* 69, 1708–1722
55 Jiang, Y. *et al.* (2018) The Role of the TGFβ Receptor Signaling Pathway in Adult Beta Cell Proliferation. *Int. J. Mol. Sci.* 19, 3136
56 Jacovetti, C. *et al.* (2015) Postnatal β-cell maturation is associated with islet-specific microRNA changes induced by nutrient shifts at weaning. *Nat. Commun.* 6, 8084
57 Hall, E. *et al.* (2018) The effects of high glucose exposure on global gene expression and DNA methylation in human pancreatic islets. *Mol. Cell. Endocrinol.* 472, 57–67
58 Kim, H. *et al.* (2020) PRMT1 Is Required for the Maintenance of Mature β-Cell Identity. *Diabetes* 69, 355–368
59 Jaafar, R. *et al.* mTORC1-to-AMPK switching underlies β cell metabolic plasticity during maturation and diabetes. *J. Clin. Invest.* 129, 4124–4137
60 Avrahami, D. *et al.* (2020) Single-cell transcriptomics of human islet ontogeny defines the molecular basis of β-cell dedifferentiation in T2D. *Mol. Metab.* 42, 101057
61 Kojima, I. *et al.* (2017) Role of the glucose-sensing receptor in insulin secretion. *Diabetes Obes. Metab.* 19, 54–62
62 Chen, C. *et al.* (2017) Human beta cell mass and function in diabetes: Recent advances in knowledge and technologies to understand disease pathogenesis. *Mol. Metab.* 6, 943–957
63 Sasaki, H. *et al.* (2021) Revisiting Regulators of Human β-cell Mass to Achieve β-cell-centric Approach Toward Type 2 Diabetes. *J. Endocr. Soc.* 5, bvab128
64 Moyce, B.L. and Dolinsky, V.W. (2018) Maternal β-Cell Adaptations in Pregnancy and Placental Signalling: Implications for Gestational Diabetes. *Int. J. Mol. Sci.* 19, 3467
65 Alarcon, C. *et al.* (2016) Pancreatic β-Cell Adaptive Plasticity in Obesity Increases Insulin Production but Adversely Affects Secretory Function. *Diabetes* 65, 438–450
66 Kluth, O. *et al.* (2015) Identification of Four Mouse Diabetes Candidate Genes Altering β-Cell Proliferation. *PLoS Genet* 11, e1005506
67 Takahashi, M. *et al.* (2020) Biphasic changes in β-cell mass around parturition are accompanied by increased serotonin production. *Sci. Rep.* 10, 4962
68 Tenenbaum, M. *et al.* (2021) The Map3k12 (Dlk)/JNK3 signaling pathway is required for pancreatic beta-cell proliferation during postnatal development. *Cell. Mol. Life Sci. CMLS* 78, 287–298

69 Rosselot, C. *et al.* (2019) Myc Is Required for Adaptive ß-Cell Replication in Young Mice but Is not Sufficient in One-Year-Old Mice Fed with a High-Fat Diet. *Diabetes* DOI: 10.2337/db18-1368
70 Keller, M.P. *et al.* (2016) The Transcription Factor Nfatc2 Regulates β-Cell Proliferation and Genes Associated with Type 2 Diabetes in Mouse and Human Islets. *PLoS Genet.* 12, e1006466
71 Sanchez-Parra, C. *et al.* (2018) Contribution of the Long Noncoding RNA H19 to β-Cell Mass Expansion in Neonatal and Adult Rodents. *Diabetes* 67, 2254–2267
72 Corrales, P. *et al.* (2021) Obesity and pregnancy, the perfect metabolic storm. *Eur. J. Clin. Nutr.* 75, 1723–1734
73 Kumar, S. *et al.* (2016) Fatty acid induced metabolic memory involves alterations in renal histone H3K36me2 and H3K27me3. *Mol. Cell. Endocrinol.* 422, 233–242
74 Hall, E. *et al.* (2014) Effects of palmitate on genome-wide mRNA expression and DNA methylation patterns in human pancreatic islets. *BMC Med.* 12, 103
75 Liu, J. *et al.* (2021) Increased Prolactin is an Adaptive Response to Protect Against Metabolic Disorders in Obesity. *Endocr. Pract.* 27, 728–735
76 Chen, K.-Y. *et al.* (2021) Maternal Plasma Lipids During Pregnancy, Insulin-like Growth Factor-1, and Excess Fetal Growth. *J. Clin. Endocrinol. Metab.* 106, e3461–e3472
77. Brooks-Worrel, B.M. *et al.* (2021) Islet Autoimmunity in Adults With Impaired Glucose Tolerance and Recently Diagnosed, Treatment Naïve Type 2 Diabetes in the Restoring Insulin SEcretion (RISE) Study. *Front. Immunol*. 12, 640251
78 Talchai, C. *et al.* (2012) Pancreatic β-Cell Dedifferentiation As Mechanism Of Diabetic β-Cell Failure. *Cell* 150, 1223–1234
79. Ye, J. *et al.* (2018) Identification of loci where DNA methylation potentially mediates genetic risk of type 1 diabetes. *J. Autoimmunity* 93, 66-75
80 Hall, E. *et al.* (2013) DNA methylation of the glucagon-like peptide 1 receptor (GLP1R) in human pancreatic islets. *BMC Med. Genet.* 14, 76
81 Karaglani, M. *et al.* (2019) Search for Pharmacoepigenetic Correlations in Type 2 Diabetes Under Sulfonylurea Treatment. *Exp. Clin. Endocrinol. Diabetes* 127, 226–233
82 Kawamori, D. *et al.* (2017) GLP-1 signalling compensates for impaired insulin signalling in regulating beta cell proliferation in βIRKO mice. *Diabetologia* 60, 1442–1453
83 Dai, C. *et al.* (2017) Age-dependent human **β** cell proliferation induced by glucagon-like peptide 1 and calcineurin signaling. *J. Clin. Invest.* 127, 3835–3844
84 Basile, G. *et al.* (2019) How, When, and Where Do Human β-Cells Regenerate? *Curr. Diab. Rep.* 19, 48
85 Frias, J.P. *et al.* (2020) Efficacy and tolerability of tirzepatide, a dual glucose-dependent insulinotropic peptide and glucagon- like peptide- 1 receptor agonist in patients with type 2 diabetes: A 12- week, randomized, double- blind, placebo- controlled study to evaluate different dose- escalation regimens. *Diabetes Obes. Metab.* 22, 938–946
86 Abdulreda, M.H. *et al.* (2016) Liraglutide Compromises Pancreatic β Cell Function in a Humanized Mouse Model. *Cell Metab.* 23, 541–546
87 Oliveira, A.G. *et al.* (2018) The Role of Hepatocyte Growth Factor (HGF) in Insulin Resistance and Diabetes. *Front. Endocrinol.* 9,
88 Vallarola, A. *et al.* (2020) A Novel HGF/SF Receptor (MET) Agonist Transiently Delays the Disease Progression in an Amyotrophic Lateral Sclerosis Mouse Model by Promoting Neuronal Survival and Dampening the Immune Dysregulation. *Int. J. Mol. Sci.* 21, 8542
89 Lund-Blix, N.A. *et al.* (2017) Infant Feeding and Risk of Type 1 Diabetes in Two Large Scandinavian Birth Cohorts. *Diabetes Care* 40, 920–927

90 Achong, N. *et al.* (2018) The physiological and glycaemic changes in breastfeeding women with type 1 diabetes mellitus. *Diabetes Res. Clin. Pract.* 135, 93–101
91 El-Badawy, A. and El-Badri, N. (2016) The cell cycle as a brake for β-cell regeneration from embryonic stem cells. *Stem Cell Res. Ther.* 7, 9
92 Qiu, W.-L. *et al.* (2017) Deciphering Pancreatic Islet β Cell and α Cell Maturation Pathways and Characteristic Features at the Single-Cell Level. *Cell Metab.* 25, 1194-1205.e4
93 Balcazar, N. *et al.* (2009) mTORC1 Activation Regulates β-Cell Mass and Proliferation by Modulation of Cyclin D2 Synthesis and Stability. *J. Biol. Chem.* 284, 7832–7842
94 Martin, D. and Grapin-Botton, A. (2017) The Importance of REST for Development and Function of Beta Cells. *Front. Cell Dev. Biol.* 5, 12
95 Henaoui, I.S. *et al.* (2017) PIWI-interacting RNAs as novel regulators of pancreatic beta cell function. *Diabetologia* 60, 1977–1986
96 Möllers, L.S. *et al.* (2021) Metabolic-endocrine disruption due to preterm birth impacts growth, body composition, and neonatal outcome. *Pediatr. Res.* DOI: 10.1038/s41390-021-01566-8
97. Vasavada, R.C. *et al.* (2006) Growth factors and beta cell replication. *Int. J. Biochem. Cell Biol.* 38, 931–950
98 Melnik, B.C. (2011) Milk signalling in the pathogenesis of type 2 diabetes. *Med. Hypotheses* 76, 553–559
99 Chen, H. *et al.* (2011) PDGF signalling controls age-dependent proliferation in pancreatic β-cells. *Nature* 478, 349–355
100 Fields, D.A. and Demerath, E.W. (2013) Human Milk Composition: Nutrients and Bioactive Factors. *Pediatr. Clin. North Am.* 60, 49–74
101 Blum, B. *et al.* (2012) Functional β-cells maturation is marked by an increase in the glucose threshold for insulin secretion and by expression of urocortin3. *Nat. Biotechnol.* 30, 261–264
102 Stolovich-Rain, M. *et al.* (2015) Weaning triggers a maturation step of pancreatic β cells. *Dev. Cell* 32, 535–545
103 Jermendy, A. *et al.* (2011) Rat neonatal beta cells lack the specialised metabolic phenotype of mature beta cells. *Diabetologia* 54, 594–604
104 Cheng, Q. *et al.* (2016) System-L amino acid transporters play a key role in pancreatic β-cell signalling and function. *J. Mol. Endocrinol.* 56, 175–187
105 Bollheimer, L.C. *et al.* (2005) Glucagon production of the rat insulinoma cell line INS-1-A quantitative comparison with primary rat pancreatic islets. *Biochem. Biophys. Res. Commun.* 330, 327–332
106 Elghazi, L. *et al.* Role of Nutrients and mTOR Signaling in the Regulation of Pancreatic Progenitors Development. *Mol. Metab.* DOI: 10.1016/j.molmet.2017.03.010
107 Stewart, A.F. *et al.* (2015) Human β-cell proliferation and intracellular signaling: part 3. *Diabetes* 64, 1872–1885
108 Wu, Y. *et al.* (2014) Dynamic expression and localization of c-MET isoforms in the developing rat pancreas. *Int. J. Clin. Exp. Pathol.* 7, 8563–8572
109 Esser, N. *et al*. (2020) Early beta cell dysfunction vs insulin hypersecretion as the primary event in the pathogenesis of dysglycaemia. *Diabetologia* 63, 2007-2021
110 Holman, R.R. *et al*. (2020) β-cell secretory dysfunction: a key cause of type 2 diabetes. *Lancet Diabetes Endocrinol* 8, 370

FIGURE LEGENDS

Figure I (text box): Molecular signature of immature neonatal ß-cell. At birth, as the pancreas continues to develop and expand, ß-cells display a significant proliferative capacity. This proliferative capacity results from the synergy of multiple signalling pathway triggered by a variety of humoral factors that converge to activate cell cycle entry and β-cell replication.

Figure 1: Signaling pathways and epigenetic reprograming involved in the nutritional switch at the suckling-weaning transition.

During the postnatal period, pancreatic ß-cells undergo a maturation process leading to an enhanced secretory capacity in response to glucose, which is accompanied by a reduced proliferative rate. This phenotypic and functional transition appears to be driven by hormonal and growth factors present in mother's milk and in the systemic circulation. This transition is also largely influenced by nutrients and mirrors the shift from a fatty acid-rich diet to a predominantly carbohydrate diet occurring at weaning. From a cellular point of view, this results in epigenetic reprogramming and in changes in the expression of multiple genes. The liver, a major organ in glucose homeostasis, participates in the cellular and molecular remodelling of ß-cell activities by adapting the production and secretion of Fetuin-A. * Genes whose activation relies on H3K4 trimethylation; † Genes whose activation relies on H3K27me3 trimethylation: Ω Genes whose activation relies on H4R3me2a trimethylation

Figure 2: Epigenetic reprogramming of mature β-cells to expand the functional β-cell mass under insulin resistance conditions

During pregnancy or obesity, compensatory mechanisms are put in place to increase the functional ß-cell mass and compensate for the increased insulin needs. Nutrients and humoral factors reactivate the epigenetic program of neonatal immature β-cells to induce cell cycle entry and β-cell expansion, permitting to maintain normoglycemia. The reprogramming of mature β-cells may or may not involve a transient loss of glucose responsiveness in proliferating β-cells. In genetically predisposed individuals, the epigenetic reprogramming fails or causes a loss of β-cell identity and dedifferentiation of insulin-secreting cells to a pancreatic multiprogenitor-like state. In this case, the amount of insulin produced is insufficient to preserve blood glucose homeostasis, resulting in the development of diabetes.

-**Outstanding Questions Box**.

- Are there differences in the mechanisms inducing β-cell mass expansion under obesity and pregnancy conditions?
- Does proliferation of reprogrammed neonatal-like β-cells always require a transient loss of glucose responsiveness?
- What is the role of intestinal bacteria and innate immune cells in the induction of β-cell epigenetic pathways during early postnatal β-cell proliferation and maturation?
- Do chronic inflammation and changes in microbiota contribute to the alteration of epigenetic pathways in β-cells
- Are defective signalling pathways elicited by nutrients and humoral factors the leading cause of altered epigenetic reprogramming in diabetes?
- Do genetic risk factors interfere with epigenetic reprogramming in diabetes patients?
- Are defective epigenetic pathways affecting neonatal BCM expansion and β-cell function in offspring of parents with T2D?
- Could the epigenetic pathways governing β-cell proliferation be modulated to expand the functional BCM in diabetic patients?

Regulation of Islet β-cell cycle

Activators that promote G1 to S phase progression play a key role in BCM expansion. This phase transition requires the inactivation of cell cycle inhibitors of the retinoblastoma protein (pRb) family (p107, p130). This is achieved by phosphorylation of pRb by cyclin/Cdk complexes including a d-type cyclin (Ccnd1, Ccnd2 or Ccnd3) and either Cdk4 or Cdk6. The kinase activity of Cdk4 or Cdk6 is negatively regulated by the level of CIP/KIP family members, including p57 (also called Cdkn1c). In newborn β-cells, Cdk4 and Cdk6 activity is stimulated upon reduction of p57 expression [92]. In mice, Cdk4 activity is enhanced after cytoplasmic retention of the Cdk4 inhibitor $p27^{kip1}$ (also called Cdkn1b) by the Forkhead Box (Fox) transcription factor FoxM1. Cdk4 and Cdk6 activity can also be potentiated by the induction of a member of the Ccnd family. Indeed, Ccnd1 and Ccnd2 appear to be key drivers of β-cell proliferation. During suckling period, the expression of the two cyclins is induced by several signaling cascades including the mTOR/raptor (mTORC1) [93], Calcineurin/nuclear factor of activated T-cells (NFATc) [78] and the c-Jun amino terminal kinase (JNK) signaling pathways [68] (**Fig. I**). The transcription factor c-Myc triggered by mTORC1 plays a major role in the control of β-cell proliferation by inducing the expression of Ccna and Ccne, two cyclins associated with either Cdk1 or Cdk2 that inactivate pRb. In addition, together with the transcriptional repressor RE-1 silencing transcription factor (REST) [94], mTORC1 contributes to neonatal β-cell immaturity by inhibiting the expression of genes involved in glucose-induced insulin secretion (GSIS) and insulin expression. Beside mTORC1, NFATc and JNK, different non-coding RNAs (ncRNAs) including microRNAs (e.g. miR-17/92 cluster, miR-194-5p, miR-181b-5p and miR-129-5p), long ncRNAs (e.g. H19) and PIWI-interacting RNAs stimulate neonatal β-cell proliferation while maintaining functional β-cell immaturity [56,71,95].

Maternal milk content promotes neonates β-cell proliferation

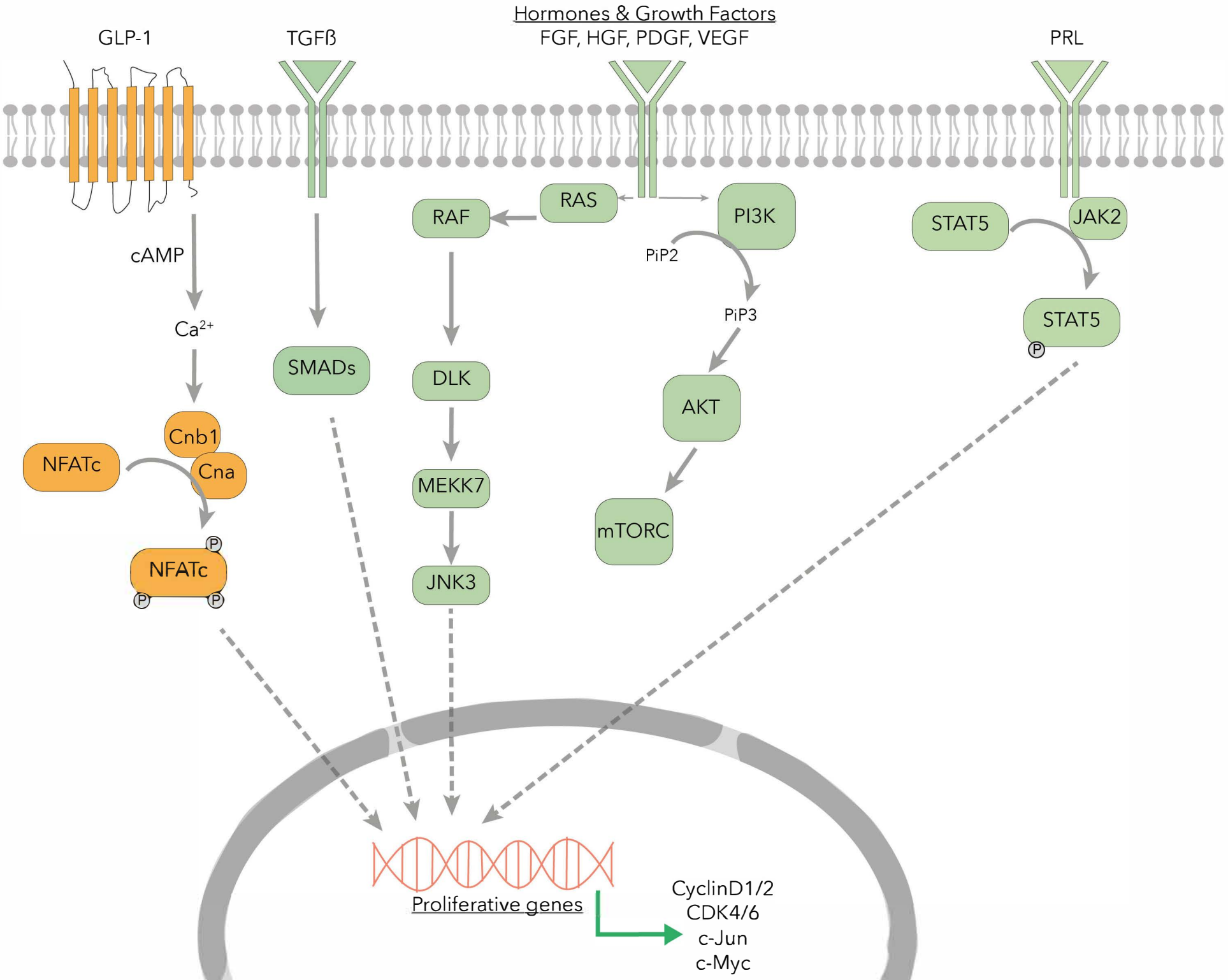
Maternal milk contains several growth factors (GF) and hormones including Fibroblast Growth Factor (FGF), Glucagon-like Peptide 1 (GLP-1), Hepatocyte Growth Factor (HGF), Insulin-like Growth Factor 1/2 (IGF-1/2), Platelet-derived Growth Factor (PDGF), Prolactin (PRL), Transforming Growth Factor (TGF) and Vascular Endothelial Growth Factor (VEGF). The concentration of these GF and hormones is elevated in the newborn blood during the suckling period, potentially exerting mitogenic effects on β-cells [96-99]. Maternal milk is also enriched in poly- and mono-unsaturated fatty acids (FA) [100]. FAs and amino-acids not only serve as fuels for stimulating insulin secretion [101], but play also a critical role in maintaining neonatal β-cell immaturity and in permitting β-cell expansion [102]. For these nutrients to exert their effects, neonatal β-cell express high levels of FA and amino acid transporters, as exemplified by Slc27a5 a FA transporter which is abundant in neonatal β-cells [103]. β-cells express also several system-L Amino acid Transporters (LATs) that allow the transport of branched-chain amino acids including leucine and isoleucine, which are insulin secretagogues [104]. LAT1 is the most abundant transporter in insulin-producing INS-1 cells, a cell line with properties similar to neonate β-cells [105], and is capable of stimulating mTorc1 signalling [104]. Newborn β-cells express also several GF and hormone receptors capable of inducing Ccnd1/2 expression through activation of signalling pathways driven by NAFTc, such as mTORC1, MAPK and JAK2/STAT5 [106-108]. The presence of these receptors is essential to allow GFs and hormones particularly abundant in the neonate islet microenvironment to modulate β-cell proliferation [97].

Functional BCM in the main types of diabetes

Type 1 diabetes (T1D), is a chronic disease defined by a drastic lack of insulin caused by autoimmune destruction of islet β-cells leading to a massive BCM reduction. Treatment mainly consists of insulin replacement by daily injections.

Type 2 diabetes (T2D) is the predominant form of diabetes and is often encountered in the context of overweight, obesity and lack of physical activity. In T2D, hyperglycemia results from β-cell dysfunction leading to insufficient insulin production to compensate for the diminished insulin sensitivity (referred to as insulin resistance) of target tissues, in particular liver, skeletal muscle and white adipose tissue. Currently, there are two hypotheses in debate to explain β-cell failure and, thereby, the development of T2D [109, 110]. The predominant hypothesis is that β-cell dysfunction occurs very early in the history of the disease. In fact, β-cell insulin secretion in response to glucose under insulin resistant conditions is already defective at the glucose intolerance stage. The alternative hypothesis points to insulin hypersecretion occurring as an adaptive response to protect against energy-induced metabolic stress as the primary cause initiating and sustaining the development of obesity and insulin resistance. β-cell failure would in this case be caused by the persistence of this insulin resistant state. The release of insufficient amounts of insulin results from defective insulin secretion in response to glucose and eventually other secretagogues which may be exacerbated by a progressive loss of BCM. To date, there is no consensus as to the cellular and molecular mechanisms underlying insulin resistance. ß-cell proliferation to compensate insulin resistance has been widely described in various obese pre-diabetic rodent models but remains controversial in humans. De-differentiation of insulin-secreting cells may also contribute to the impairment of ß-cell function associated with T2D [74]. T2D treatment varies according to the pathophysiological condition of the patients and their insulin sensitivity status. Oral anti-diabetic drugs that improve insulin sensitivity are typically used with or without the administration of exogenous insulin.

Gestational diabetes refers to an increase in blood glucose levels that occurs during pregnancy. In pregnant women, insulin sensitivity of peripheral tissues decreases to minimize insulin-regulated lipogenesis and thus divert fatty acids into the bloodstream to meet the nutritional needs of the fetus. Under normal conditions, compensatory mechanisms involving an increase in maternal ß-cell mass and function are triggered to maintain glucose homeostasis.


Hormones & Growth Factors
FGF, HGF, PDGF, VEGF
GLP-1
TGFß
PRL
cAMP
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SMADs
RAS
RAF
DLK
MEKK7
JNK3
PI3K
PiP2
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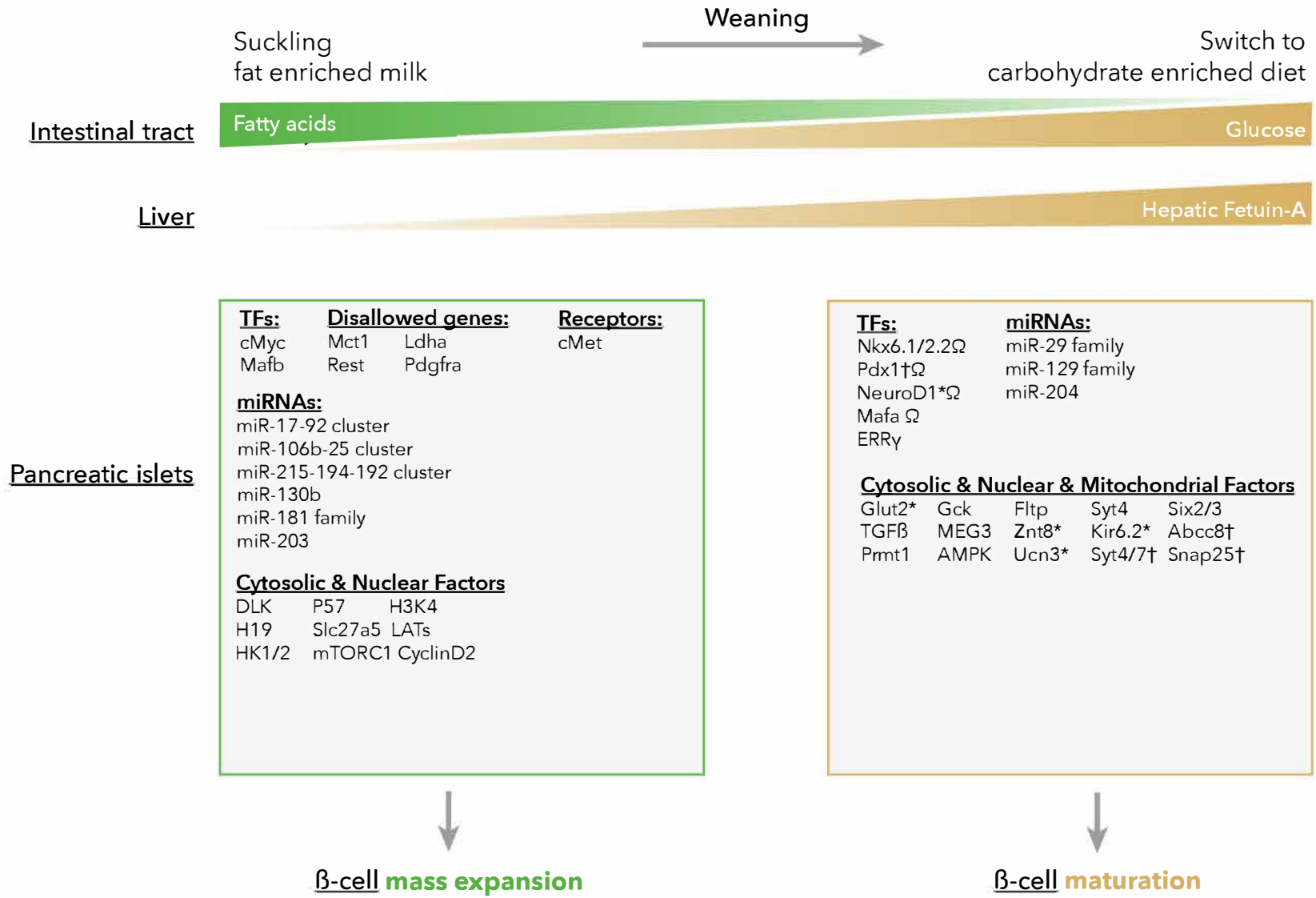
c-Jun
c-Myc


Weaning
Suckling
fat enriched milk
Switch to
carbohydrate enriched diet
Intestinal tract
Fatty acids
Glucose
Liver
Hepatic Fetuin-A
Pancreatic islets
TFs:
cMyc
Mafb
Disallowed genes:
Mct1
Ldha
Rest
Pdgfra
Receptors:
cMet
miRNAs:
miR-17-92 cluster
miR-106b-25 cluster
miR-215-194-192 cluster
miR-130b
miR-181 family
miR-203
Cytosolic & Nuclear Factors
DLK
P57
H3K4
H19
Slc27a5
LATs
HK1/2
mTORC1
CyclinD2
TFs:
Nkx6.1/2.2Ω
Pdx1†Ω
NeuroD1*Ω
Mafa Ω
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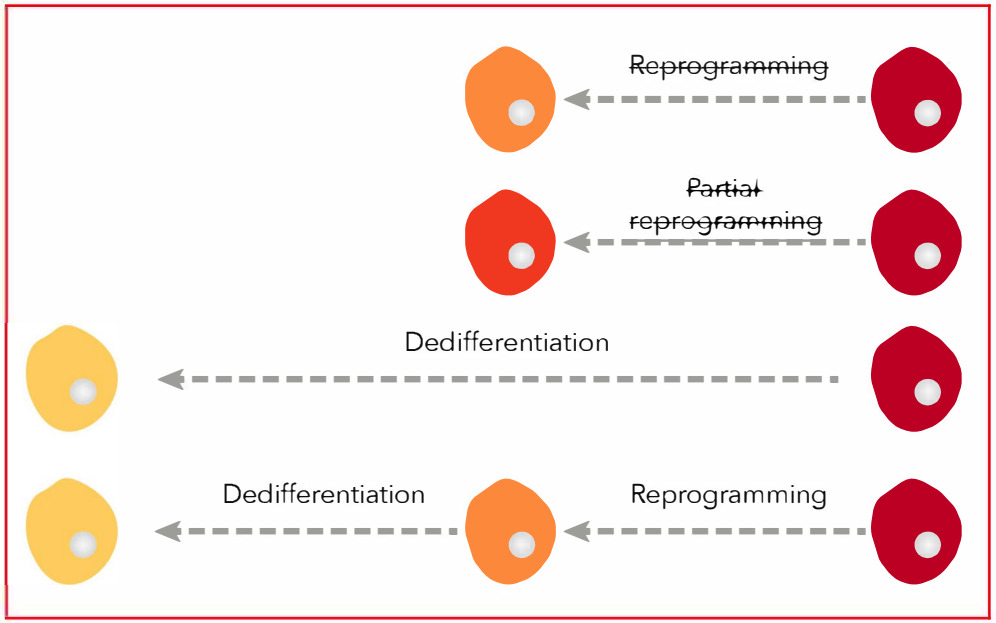
miR-29 family
miR-129 family
miR-204
Cytosolic & Nuclear & Mitochondrial Factors
Glut2*
Gck
Fltp
Syt4
Six2/3
TGFß
MEG3
Znt8*
Kir6.2*
Abcc8†
Prmt1
AMPK
Ucn3*
Syt4/7†
Snap25†
ß-cell mass expansion
ß-cell maturation

Pregnancy & Obesity

With genetic variants & epigenetic alterations

Defective epigenetic mechanisms

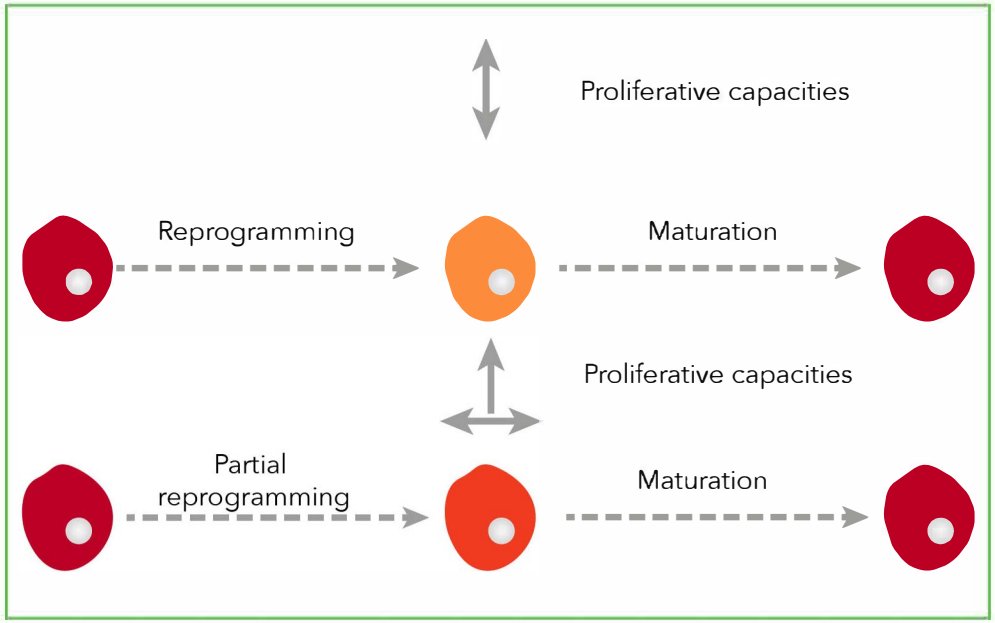


Impaired functional ß-cell mass expansion

Diabetes

Without genetic predisposition & epigenetic marks

Adaptive epigenetic mechanisms driven by GFs, hormones, fatty acids and amino acids



Functional ß-cell mass expansion

Normoglycemia