Transgenic and Knockout Mice in Diabetes Research: Novel Insights into Pathophysiology, Limitations, and Perspectives

Insulin resistance and type 2 diabetes are serious public health threats. Although enormous research efforts have been focused on the pathogenesis of these diseases, the underlying mechanisms remain only partly understood. Here we review mouse phenotypes resulting from inactivation of molecules responsible for the control of glucose metabolism that have led to novel insights into insulin action and the development of insulin resistance. In addition, more sophisticated strategies to manipulate genes in mice in the future are presented.

Type 2 diabetes mellitus is the most common endocrine disease, affecting ~5% of Western populations with steadily increasing incidence (77). Today, more than 18 million Americans (~6.3% of the U.S. population) suffer from diabetes, an important risk factor for cardiovascular disease, which is the main cause of death worldwide (43, 55). Diabetes is the most common cause of blindness among working-age adults, the most common cause of renal failure, and the sixth most common cause of death in the U.S. (99a). The rising prevalence of type 2 diabetes worldwide is mainly the consequence of the drastically increasing prevalence of obesity (35).

Epidemiological and twin studies have clearly indicated a genetic predisposition for the development of type 2 diabetes (38, 42, 74, 82), which is most likely of varying penetrance and of polygenic nature (38). It could be demonstrated that the fundamental dysfunction in type 2 diabetes is insulin resistance, which comprises both the failure of insulin to stimulate glucose uptake in skeletal muscle and adipose tissue and its inability to suppress hepatic glucose production sufficiently (71, 73). To define potential genetic defects, research over the past two decades has focused on the identification and characterization of molecules involved in the insulin-signaling pathway, and much of the current progress in our understanding of insulin action and insulin resistance has been made with the use of mouse models with genetic modifications in this and intercommunicating pathways (Table 1).

Insulin exerts its diverse biological effects by binding to and activating the membrane-bound insulin receptor (IR), thereby initiating the insulin signal transduction cascade (for details, see http://stke.sciencemag.org/cgi/cm/CMP_12069). IRs are not only present in the classical insulin target tissues, i.e., adipose tissue, skeletal muscle, and liver, but are also widely expressed throughout the organism in tissues such as pancreas (45), the central nervous system (CNS) (72), lymphatic cells (11, 17), and kidney (6, 62). Signals initiated by the activated IR result in the diverse biological effects of insulin, such as the stimulation of glucose transport, the inhibition of gluconeogenesis, the stimulation of protein and lipid synthesis, and the regulation of gene transcription. Because the roles of many components of the signaling pathway were initially characterized in vitro, more recent research has focused on the analysis of insulin action in vivo by generating mice with targeted disruptions of genes for these components. Since the pattern of inheritance of type 2 diabetes points to a disease of polygenic nature, not only single gene-deficient mice but also models with combined genetic disruptions have been created in an attempt to represent the polygenic nature of the disease. New techniques have also become available for conditional gene inactivation in mice. This is particularly helpful if conventional knockout of a gene results in embryonic lethality, preventing the analysis of gene function in the adult. Conditional mutagenesis enables the inactivation of a gene of interest in a specific tissue (92, 93, 99) or in a temporally controlled manner, thereby providing new insights into the contribution of a gene to a complex physiological phenotype such as insulin resistance.

Knockout Models with Inactivation of Insulin-Signaling Molecules

The first knockout models of insulin resistance aimed at the disruption of major molecules in IR signaling. The successful generation of viable, heterozygous IR knockout mice showed that 50% of IR expression is sufficient for the maintenance of physiological blood glucose concentrations. In contrast, homozygous IR-deficient mice rapidly
develop diabetic ketoacidosis and die within 3–7 days after birth (2, 49), showing the indispensability of IR for the control of glucose metabolism.

**Skeletal muscle and insulin resistance**

Because insulin resistance in skeletal muscle is one of the earliest detectable defects in type 2 diabetic patients, muscle-specific insulin receptor knockout (MIRKO) mice have been generated as a model for muscle-specific insulin resistance. MIRKO mice displayed elevated fat mass, serum triglycerides, and free fatty acids, indicating that insulin resistance in muscle contributes to the altered fat metabolism associated with type 2 diabetes (20).

<table>
<thead>
<tr>
<th>Model</th>
<th>Viable</th>
<th>DM</th>
<th>Miscellaneous</th>
<th>Reference</th>
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<tr>
<td>IR+/–</td>
<td>yes</td>
<td>no</td>
<td></td>
<td>2</td>
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<tr>
<td>IR–/–</td>
<td>yes</td>
<td>yes</td>
<td>Pups die within 7 days after birth from diabetic ketoacidosis</td>
<td>49</td>
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<td>β-IRKO</td>
<td>yes</td>
<td>yes</td>
<td>Reduced fat mass, protection against obesity and related glucose intolerance, altered adipose tissue histomorphology</td>
<td>59</td>
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<tr>
<td>FIRKO</td>
<td>yes</td>
<td>no</td>
<td></td>
<td>13–15</td>
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<td>LIRKO</td>
<td>yes</td>
<td>yes</td>
<td>Progressive liver failure</td>
<td>75</td>
</tr>
<tr>
<td>MIRKO</td>
<td>yes</td>
<td>no</td>
<td>Adiposity due to redistribution of substrates to adipose tissue</td>
<td>20</td>
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<tr>
<td>NIRKO</td>
<td>yes</td>
<td>no</td>
<td>Normal brain development; mild insulin resistance, diet-sensitive obesity; dysregulation of luteinizing hormone</td>
<td>21</td>
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<tr>
<td>MIRKO and MinIGF-1R</td>
<td>yes</td>
<td>yes</td>
<td>Depletion of fat stores; mild impairment of glucose metabolism; growth retardation; cardiac hypertrophy</td>
<td>34</td>
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<tr>
<td>MIRKO and FIRKO</td>
<td>yes</td>
<td>no</td>
<td></td>
<td>63</td>
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<td>Glut4–/–</td>
<td>yes</td>
<td>no</td>
<td>Insulin resistance; hypertension, elevated serum triglycerides, impaired endothelium-dependent vascular relaxation</td>
<td>103</td>
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<tr>
<td>MGlut4KO</td>
<td>yes</td>
<td>yes</td>
<td>Insulin resistance; hypertension, elevated serum triglycerides, impaired endothelium-dependent vascular relaxation</td>
<td>1a</td>
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<tr>
<td>FGlut4KO</td>
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<td>no</td>
<td>Insulin resistance; hypertension, elevated serum triglycerides, impaired endothelium-dependent vascular relaxation</td>
<td>1, 98</td>
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<td>IRS-2–/–</td>
<td>yes</td>
<td>yes</td>
<td>Lipotrophy</td>
<td>58, 89</td>
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<td>IRS-1–/– and IRS-3–/–</td>
<td>yes</td>
<td>yes</td>
<td>Single disruption of either gene does not produce diabetes</td>
<td>3, 98</td>
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<td>IRS-1+/– and IR+/–</td>
<td>yes</td>
<td>yes</td>
<td>Improved insulin sensitivity vs. IR–/–</td>
<td>19</td>
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<td>Foxo1–/–</td>
<td>yes</td>
<td>no</td>
<td>Normal development of β-cells; decreased expression of Glut2 and GK in β-cells</td>
<td>81</td>
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<td>IRS-1–/– and β-cell GKKO</td>
<td>yes</td>
<td>yes</td>
<td>Major defects only after pharmacological challenge</td>
<td>34, 67</td>
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<tr>
<td>IRS-1–/– and IR+/–</td>
<td>yes</td>
<td>yes</td>
<td>Improved insulin sensitivity vs. IR–/–</td>
<td>81</td>
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<td>β-IGF-1RKO</td>
<td>yes</td>
<td>yes</td>
<td>Normal development of β-cells; decreased expression of Glut2 and GK in β-cells</td>
<td>60</td>
</tr>
<tr>
<td>PPAR-α–/–</td>
<td>yes</td>
<td>no</td>
<td>Major defects only after pharmacological challenge</td>
<td>60</td>
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<td>PPAR-γ–/–</td>
<td>yes</td>
<td>no</td>
<td>Mild insulin resistance</td>
<td>56</td>
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<td>PPAR-γ2–/–</td>
<td>yes</td>
<td>no</td>
<td>Reduction of white adipose tissue; insulin resistance</td>
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<td>FPPAR-γKO</td>
<td>yes</td>
<td>yes</td>
<td>Loss of fat; hyperlipidemia, fatty liver</td>
<td>102</td>
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<td>C/EBP-α–/–</td>
<td>yes</td>
<td>no</td>
<td>Death from hypoglycemia within 8 h of birth</td>
<td>100</td>
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DM, diabetes mellitus; IR, insulin receptor; KO, knockout; dn, dominant negative; β, β-cell specific, f, fat/adipose tissue specific; L, liver specific; M, muscle specific; N, neuron specific; IGF-1R, insulin-like growth factor I receptor; Glut4, glucose transporter 4; IRS-1/2/3, insulin receptor substrate-1/2/3; Foxo1, forkhead transcription factor O1; GK, glucokinase; PPAR, peroxisome proliferator-activated receptor; C/EBP, CCAAT enhancer-binding protein.
Additionally, Kim et al. (53) demonstrated that IR deficiency in muscle promotes redistribution of substrates to adipose tissue, thereby contributing to increased adiposity in MIRKO mice. Surprisingly, blood glucose, serum insulin, and glucose tolerance were normal in these mice, initially leading to the interpretation that tissues other than muscle are more essential for insulin-regulated glucose disposal than previously assumed. However, Zisman et al. (103) disrupted the gene for the glucose transporter 4 (Glut4), which mediates glucose transport in response to insulin, selectively in mouse muscle tissue. They found that Glut4-mediated glucose uptake in muscle was indeed essential for the maintenance of normal glucose homeostasis. Consistently, mice with a combined muscle-specific functional inactivation of IR and the closely related insulin-like growth factor receptor (IGF-1R) also display a complete type 2 diabetic phenotype, implicating a compensatory role of IGF-1R for insulin's stimulation of glucose transport via Glut4 in muscle (34).

The role of adipose tissue in muscle-specific IR deficiency was addressed in mice with a targeted inactivation of IR-mediated signaling in both muscle and adipose tissue (63). These mice did not become diabetic despite peripheral insulin resistance and a mild impairment of β-cell function. Together, these data suggest that there may be a critical threshold in whole body insulin resistance that finally leads to a diabetic phenotype. Moreover, this insulin resistance might result in excessive demands on the pancreatic β-cells to secrete insulin, leading to consecutive decompensation of these cells. Although dysfunction of the pancreatic β-cell is undoubtedly rightly considered as an important defect in the pathogenesis of type 2 diabetes, its exact relationship to insulin resistance remained unclear.

**Role of the β-cell in impaired glucose tolerance**

Mice with a specific disruption of the IR gene in β-cells show a selective loss of insulin secretion in response to glucose and a progressive impairment of glucose tolerance (39), indicating that insulin stimulates its own secretion via IRs on β-cells, thus playing an important functional role in glucose sensing by the pancreatic β-cell. Therefore, defects in insulin signaling at the level of the β-cell may contribute to the observed alterations in insulin secretion in type 2 diabetes. Interestingly, a β-cell-specific IGF-1R knockout model also underscored a role for this receptor in the control of glucose-stimulated insulin secretion and glucose tolerance (60). The interplay between insulin resistance and insulin secretory defects has also been addressed by the generation of mice deficient for insulin receptor substrate 1 (IRS-1) and β-cell glucokinase (GK) (98). The heterozygous β-cell GK knockout is characterized by decreased insulin secretion in response to glucose (3), whereas IRS-1-deficient mice are insulin resistant but do not develop overt diabetes (1). In contrast to either individual mutation, the double-knockout mice developed a diabetic phenotype, demonstrating that the combination of individual minor defects in insulin action or insulin secretion can cause overt diabetes.

**Insulin resistance in the brain**

The liver-specific insulin receptor knockout (LIRKO) mouse, as expected, exhibits a dramatic phenotype with severe insulin resistance and progressive liver failure (75), mirroring the critical role of insulin signaling in liver for regulating glucose homeostasis and maintaining normal hepatic function. However, IRs and insulin-signaling proteins are not exclusively expressed in classic insulin target tissues; they are also widely distributed throughout the CNS. CNS-specific disruption of the IR gene (NIRKO) revealed an important role of IRs in the regulation of energy disposal, fuel metabolism, and reproduction (21). It has very recently been shown in conventional IR-deficient mice that combined restoration of IR function selectively in brain, liver, and pancreatic β-cells rescues these mice from neonatal death, prevents diabetes in a majority of animals, and restores adipose tissue content, lifespan, and reproductive function. Interestingly, IR substitution either limited to brain or liver and pancreatic β-cells was sufficient to prevent neonatal death but not lipatrophic diabetes, leading to the surprising finding that IR signaling in nontypical insulin target tissues like the brain seems to be crucial to maintain fuel homeostasis and prevent diabetes (84).

**Disruption of IRSs**

Aside from IR knockouts, various molecules of the signal transduction pathway initiated by IR have been disrupted, such as IRS-1, which was believed to be the principal substrate for the IR and IGF-1R. Mice deficient for IRS-1 exhibit impaired glucose tolerance and a decrease in insulin/IGF-1-stimulated glucose uptake in vivo and in vitro, thereby providing a mouse model of genetically determined insulin resistance (1, 89). Additionally, elevated blood pressure and plasma triglyceride levels were observed, as well as impaired endothelium-dependent vascular relaxation (1), indicating that insulin resistance plays an important role in the clustering of coronary risk factors leading to accelerated atherosclerosis. The residual insulin/IGF-1 action in IRS-1-deficient mice correlated with the existence of an alternative tyrosine-phosphorylated protein (IRS-2) (5, 85), which is also capable of...
activating the signaling cascade. Surprisingly, disruption of IRS-2 impaired peripheral insulin signaling and pancreatic β-cell function, resulting in progressive deterioration of glucose homeostasis. This phenotype results from insulin resistance in liver and skeletal muscle and β-cell dysfunction (57). Succeeding studies indicated tissue-specific functions for IRS-1 and IRS-2 in mediating the metabolic effects of insulin in vivo. IRS-1 having a major role in skeletal muscle and IRS-2 in liver, muscle, adipose tissue, pancreatic β-cells, and reproductive tissue (22). Interestingly, mice with a double knockout of IRS-1 and the subsequently identified IRS-3 (65, 66) displayed a phenotype of early-onset severe lipatroph associated with hyperglycemia, hyperinsulinemia, and decreased plasma levels of the anorexigenic hormone leptin. The IRS-1/IRS-3 double-knockout phenotype in mice (64) might mimic the situation in humans better than the single IRS-1 knockout, since Björnholm et al. (12) demonstrated that no functional IRS-3 homolog is present in humans that could compensate for defects in IRS-1 signaling. The concept that several subclinical genetic alterations in insulin action can synergize to result in overt diabetes was tested in mice double heterozygous for IR and IRS-1 alleles with a ~50% reduction in expression of either protein (19). In these mice, the combined genetic defects led to aggravation of insulin resistance with 5- to 50-fold elevated plasma insulin levels and respective β-cell hyperplasia, and ~40% of the animals developed overt diabetes at the age of 4–6 mo. This mouse model of type 2 diabetes, in which diabetes arises in an age-dependent manner from the interaction between two genetically determined, subclinical defects in the insulin-signaling cascade, demonstrates the important role of epistatic interactions in the pathogenesis of common diseases with non-Mendelian genetics. Moreover, this model has been used successfully to further modifying C57BL/6 loci regulating insulin sensitivity (4).

The role of adipose tissue

The strong association between the world-wide epidemic of obesity and dramatically increasing prevalence of insulin resistance and type 2 diabetes has prompted recent research to focus on the mechanisms linking adipose tissue to whole body insulin sensitivity, β-cell function, and overall glucose metabolism. Newborn mice with IR deficiency show a marked reduction in white adipose tissue mass, pointing to a role for IR in the regulation of adipocyte growth and differentiation (2, 49). Mice with an adipose tissue-specific knockout of the IR (FIRKO) also display a reduced fat mass, loss of the normal correlation of plasma leptin and body weight, and protection against both obesity and obesity-related glucose intolerance (13). Interestingly, they also exhibit an 18% extended lifespan (14). Strikingly, whereas adipocytes of control mice exhibit a bell-shaped size distribution, adipocytes of FIRKO mice demerge into groups of small and large cells. Interestingly, these differently sized cells also show different expression of fatty acid synthase and the transcription factors CCAAT enhancer binding protein (C/EBP)-α as well as sterol regulatory element-binding protein 1 (SREBP1) (15, 16). Thus inactivation of the IR revealed a previously unrecognized heterogeneity of adipose tissue. Consistent with the phenotype of Glut4-null mice, which display a depletion of fat stores (51), the selective disruption of Glut4 in adipose tissue caused an impaired glucose transport, followed by development of insulin resistance (1a). These data clearly assign adipocytes an important role in lipid storage, development of obesity, and regulation of glucose homeostasis. Recent studies have identified a family of transcription factors, the Foxos, as effectors of insulin signaling in diverse cell types (reviewed in Ref. 54). Foxo1 is present in the nucleus, where it activates gene transcription; upon insulin stimulation, the transcription factor is phosphorylated and excluded from the nucleus, thus interfering with its function as a transcriptional activator. One example of Foxo1-mediated insulin action is the insulin-induced suppression of gluconeogenesis, because unphosphorylated Foxo1 induces transcription of glucose-6-phosphatase, a key gluconeogenic enzyme (80). Double-heterozygous Foxo1/IR-deficient mice exhibited improved insulin sensitivity compared with insulin-resistant heterozygous IR knockouts (81). This phenomenon is the consequence of a complex mechanism involving decreased glucose production, increased β-cell performance, and expression of insulin-sensitive genes in adipose tissue (reviewed in Ref. 32). Puigserver et al. (90) have shown that Foxo1 is coactivated by peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1α (PGC-1α) in a manner that is inhibited by insulin stimulation, thereby linking insulin-mediated regulation of transcription to PGC-1.

Transgenic and Knockout Models in Other Pathways Regulating Insulin Action

Inactivation of PPARs

It has been shown that the abnormal accumulation of lipids in tissues other than adipose adversely affects insulin sensitivity (27, 52, 86, 87), indicating a complex system for the comprehensive control of lipid and glucose homeostasis. The key coordinators in this metabolic axis are members of the
nuclear hormone receptor superfamily (31). Among those, PPARs respond to small lipid agents (28), e.g., dietary fatty acids, and contribute a key mechanism in the regulation of lipid and glucose metabolism (reviewed in Ref. 91). The important physiological role of the PPARs, i.e., PPAR-α, -β/δ, and -γ, was deduced from findings identifying the PPARs as primary targets of two key classes of synthetic compounds that have been used in the successful treatment of diabetes and dyslipidemia. In particular, thiazolidinedione (TZD) insulin sensitizers are potent and specific PPAR-γ ligands and activators (68). Fibrates have predominant activity as PPAR-α agonists, favorably affecting serum lipid levels (78). Diverse knockout models have been created to study the function of the single PPAR isoforms in vivo.

Consistent with the concept that PPAR-α is the member of the PPAR family that mediates cellular lipid utilization, pharmacological inhibition of cellular fatty acid flux in mice lacking PPAR-α caused massive hepatic and cardiac lipid accumulation, hypoglycemia, and death in 100% of male and 25% of female animals, demonstrating a pivotal role for PPAR-α in lipid and glucose homeostasis in vivo. Nevertheless, there was no major phenotypic defect detectable in PPAR-α knockouts without pharmacological challenge (26, 67). Murolo et al. showed that skeletal muscle of PPAR-α-deficient mice exhibited only minor changes in fatty acid homeostasis, and even mRNA expression of known PPAR-α target genes in muscle tissue was not significantly affected (79). They proposed that this finding might be explained by high levels of PPAR-β/δ compensating for the lack of PPAR-α, suggesting redundancy in the functions of PPARs as transcriptional regulators of fatty acid homeostasis.

In contrast to the embryonic lethality of homozygous PPAR-γ knockouts (8), mice deficient only for the PPAR-γ2 isoform survived, exhibiting an overall reduction in white adipose tissue mass, less lipid accumulation, and decreased expression of adipogenic genes in adipose tissue (102). Consistently, embryonic fibroblasts of PPAR-γ2 knockout showed a dramatically reduced capacity for adipogenesis in vitro (102). In addition, insulin sensitivity was impaired in these mice, with decreased expression of IRS-1 and Glut4 in skeletal muscle, but TZDs were able to normalize this insulin resistance (102). In contrast, heterozygous PPAR-γ knockouts show reduced disposition to insulin resistance (56). Strikingly, this phenotype is blunted by treatment with a synthetic PPAR-γ ligand of the TSD class, indicating that optimal levels of PPAR-γ activity are crucial for its beneficial effects (101). To address these obvious incongruities, PPAR-γ has been selectively disrupted in liver, adipose tissue, and muscle (31, 39, 46, 47, 83). Briefly, although adipose tissue appears to be the main site of TZD action, the conditional knockouts highlight important functions for muscle and liver PPAR-γ in the control of body composition and insulin sensitivity. PPAR-γ deficiency in adipose tissue leads to progressive loss of fat, hyperlipidemia, fatty liver, and accompanying hepatic insulin resistance (46). The mice can maintain normal whole body glucose homeostasis and normal insulin sensitivity only as long as some adipose tissue is present. These studies identify a molecular link between blood glucose homeostasis and lipid metabolism, providing a genetic basis for the observed phenotypic correlation between obesity and type 2 diabetes mellitus.

Lessons from other models

Besides PPAR-γ, C/EBP-α is also a major regulator of adipocyte differentiation. C/EBP-null mice fail to develop white adipose tissue and to store hepatogenic glycogen, leading to death from hypoglycemia within 8 h after birth (100). In cells ectopically expressing PPAR-γ, the development of insulin responsive glucose uptake in vitro correlates with C/EBP-α expression, suggesting that although PPAR-γ is sufficient to trigger the adipogenic program, C/EBP-α is required for establishment of insulin-sensitive glucose transport (29). Consistent with these findings, Cario et al. (23) demonstrated that adipocyte hyperplasia observed in MIRKO mice is accompanied by increased expression of C/EBP-α, confirming the importance of molecules that alter adipose tissue mass ("WAT plasticity") in the maintenance of whole body insulin sensitivity. SREBP-1 belongs to a family of transcription factors involved in cholesterol and fatty acid metabolism (18), and it has been suggested that it mediates the effects of insulin on hepatic genes involved in glucose and lipid metabolism (7, 37, 48, 96, 97). Overexpression of SREBP-1c specifically in the liver of diabetic mice markedly decreased hyperglycemia due to increased expression of glucokinase and lipogenic enzyme gene, and it repressed expression of phosphoenolpyruvate carboxykinase, a key enzyme of the gluconeogenic pathway, indicating a major role of SREBP-1c in the control of glucose homeostasis by insulin (10).

On the basis of these data, the emerging picture is of a complex interaction of IR-mediated signaling and pathways involving nuclear hormone receptors, physiologically linking systemic glucose homeostasis, lipid metabolism, and adipocyte biology.

Future Perspectives

The employment of even more sophisticated strategies in conditional gene targeting may overcome these limitations in the future. Conditional
The inactivation of genes is mainly achieved by the Cre-loxP system. The bacteriophage P1-derived Cre recombinase excises a segment of genomic DNA, which has been flanked with specific recognition sites ("loxP sites") by genetic engineering. The Cre recombinase gene and additional DNA elements that control its expression are also introduced into the genome of the host animal. The onset of the conditional inactivation of the gene can be triggered at specific developmental stages by a temporally controlled Cre expression (70). Presently, the inducible systems existing to control Cre recombinase activity are either at the transcriptional or the posttranslational level. Cell type-specific transcriptional control of Cre can be achieved by the tet systems, in which the activity of almost silent gene promoters can be enhanced manyfold through binding of inducer proteins that are regulated by the antibiotics tetracycline or doxycycline (40). In the tet-off system, transcription is abrogated in the presence of doxycycline, since the reverse tetracycline controlled activator rTA requires doxycycline binding to activate transcription (41) (FIGURE 1A). In both systems, the genomic integration site of the tetO promoter driving the transgene of interest is critical. When endogenous enhancers are located in close environment to the tetO promoter, the promoter can be activated even in the absence of the active

![Diagram](https://example.com/diagram.png)

**FIGURE 1. Inducible systems for conditional inactivation of genes**

A: when employing the tet-on system, transcription of Cre recombinase is induced by administration of doxycycline (Dox), which binds to the reverse tetracycline-controlled activator (rTA). B: when Cre is fused to the ligand-binding domain (LBD) of the estrogen receptor (ER), the recombinase is inactivated by heat shock proteins (HSPs) bound to the ERLBD. After addition of tamoxifen (Tam), the fusion protein becomes activated and can mediate excision of a loxP-flanked target gene in the nucleus.
transcriptional activators, resulting in leaky expression of the transgene. A recently described integration site for the tetO promoter offers an alternative approach. A bidirectional tetO promoter (9) in transgenic LC-1 mice (94) drives the expression of Cre and luciferase, respectively, and can be tightly regulated by transactivating units of both the tet and tet-off systems.

In the posttranslational regulation, a fusion protein between Cre and the ligand-binding domain (LBD) of a steroid hormone receptor can be expressed from any given cell type-specific promoter. In the absence of steroid hormone, the LBDs are bound by heat shock proteins, which inactivate recombinase activity, possibly by steric hindrance. Administration of the steroid hormone, the fusion protein becomes activated and can mediate excision of a loxP-flanked target gene in the nucleus (Figure 1B). To generate a posttranslational system of Cre fused to the LBD of the estrogen receptor (ER), single-amino-acid substitutions were introduced in the ER-LBD to prevent its activation by the endogenous hormone 17β-estradiol (E2) but maintain its binding capacities for synthetic steroids such as 4-hydroxytamoxifen (33). During recent years, numerous transient gene mouse strains were generated that express the Cre fusion protein ubiquitously (95) or restricted to several tissues, for example muscle or brain (25, 36, 58, 69). Crossing these inducible Cre mouse strains to mice carrying conditional alleles of insulin-signaling molecules allows the role of those molecules in different tissues to be distinguished, as for example the effect of insulin action in β-cell development versus roles in the insulin secretion pathway in adult mice.

However, conditional mutagenesis also has its limitations. The generation of loxP-flanked alleles is often time consuming, or of limited value, due to the existence of multiple copies of the gene and/or pseudogenes in the genome in which undesired targeting events can occur. To overcome such limitations, the recently described RNA interference (RNAi) method may offer feasible alternatives (22). Small double-stranded hairpin RNA molecules (shRNAs) complementary to a part of the messenger RNA of the gene of interest are processed by the RNAi machinery to become unwound by an RNA helicase and subsequently incorporated as single-stranded siRNAs into the RISC complex that guides this complex to the mRNA (76). Ongoing investigations in the RNAi field like the conditional expression of shRNAs using the Cre/loxP system (50) will provide a useful and fast approach in the toolkit of reverse genetics.

Conclusions

New concepts for the molecular basis of insulin action and insulin resistance have emerged thanks to the availability of techniques for conditional mutagenesis. As a result, to the view that insulin resistance of various classical and nonclassical target tissues can combine to cause the complex phenotype of insulin resistance and type 2 diabetes. Nevertheless, the conclusions derived from these experiments are limited by the fact that, even in the tissue- and cell-type-specific knockouts, gene inactivation is effective throughout development. Therefore, 1) there are probably compensatory mechanisms for the loss of individual proteins, and 2) it may be difficult to distinguish phenotypes arising from developmental defects from those resulting from impaired signaling.

In summary, mouse models with single genetic defects can yield important information about the action of insulin in glucose metabolism but may not always match the human disease states. Thus, it is critical that new, relevant animal models employing more sophisticated strategies for gene inactivation be developed to extend our understanding of the mechanisms underlying type 2 diabetes and obesity.

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References


