

A Genetic Switch in Pancreatic β -Cells

Implications for Differentiation and Haploinsufficiency

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Heterozygous mutations in the genes encoding transcriptional regulators hepatocyte nuclear factor (HNF)-1 α and HNF-4 α cause a form of diabetes known as maturity-onset diabetes of the young (MODY). Haploinsufficiency of HNF-1 α or HNF-4 α results in MODY because of defective function of pancreatic islet cells. In contrast, homozygous null mutations in mouse models lead to widespread and profound gene expression defects in multiple cell types. Thus, it is not surprising that HNF-1 α function is now known to have distinct properties in pancreatic β -cells. It controls a complex tissue-selective genetic network that is activated when pancreatic cells differentiate, and allows these cells to maintain critical specialized functions. The network contains an indispensable core component formed by a positive cross-regulatory feedback circuit between HNF-1 α and HNF-4 α . This type of circuit configuration can exhibit a switch-like behavior with two stable states. In the default active state, it can serve to perpetuate network activity in differentiated β -cells. However, the loss of one HNF-1 α or HNF-4 α allele can increase the probability that the feedback circuit is permanently switched off, resulting in decreased expression of all four alleles selectively in β -cells. Such a model can serve to rationalize key aspects of the pathogenic mechanism in MODY. *Diabetes* 51:2355–2362, 2002

The genetic analysis of mendelian forms of diabetes has produced a major breakthrough in our understanding of the transcriptional programs needed for differentiated β -cells to function properly. During a period of <3 years, mutations in five genes encoding transcriptional regulators were found to cause early-onset autosomal dominant diabetes (also known as maturity-onset diabetes of the young [MODY]) (Table 1) (1–5). The MODY3 gene, encoding an atypical homeodomain protein named hepatocyte nuclear factor (HNF)-1 α , was incriminated in its etiology through a positional cloning strategy (2,6). HNF-1 α had been identi-

fied several years before, but despite a wealth of information on its function in regulating liver enriched genes, it had not been regarded as a candidate gene for diabetes (7). The identification of HNF-1 α led immediately to the recognition that the MODY1 gene encoded HNF-4 α , a regulator of HNF-1 α (1). Because diabetes in both MODY1 and MODY3 results from a severe insulin secretory defect (6,8–10), these genetic findings indicated that HNF-1 α and HNF-4 α have an unanticipated role in controlling β -cell function (1,2). Shortly after these findings, a candidate gene approach led to the recognition that mutations in further β -cell transcriptional regulator genes caused MODY: IPF-1(MODY 4), TCF-2 (encoding HNF-1 β , MODY 5), and NeuroD-1 (3–5) (Table 1).

The identification of HNF-1 α and HNF-4 α as MODY genes has raised several fundamental questions. What are the in vivo genetic targets and the basic biological processes that are controlled by these transcriptional regulators in β -cells? How can mutations in genes known to regulate transcription in diverse cell types result in a phenotype that is largely restricted to pancreatic cells? Why are heterozygous mutations sufficient to cause the disease, and why is a genetically determined phenotype delayed until the second to fourth decade of life? On the other hand, it was assumed that these and possibly other transcriptional regulators involved in MODY participated in a common β -cell regulatory pathway, but there was no evidence to define the nature of the pathway in this particular cell type. Clearly the answers to these questions will reveal molecular mechanisms that are central in the control of differentiated β -cell function.

The molecular genetics of MODY has been the subject of several recent reviews (11–14). The purpose of this article is to discuss recent findings reported by several laboratories that have uncovered key genetic interactions of HNF-1 α and HNF-4 α in pancreatic β -cells. This information is analyzed to propose a model for the haploinsufficient mechanism in MODY.

HNF-1 α and HNF-4 α deficiency causes dysfunction of pancreatic β -cells. Humans with heterozygous HNF-1 α and HNF-4 α mutations typically develop diabetes during the second to fourth decade of life (1,6,8,13,15). Diabetes results from abnormal glucose-induced insulin secretion, rather than defective insulin action or hepatic glucose output (6,8,9,16). The β -cell defect cannot be ascribed solely to reduced cell mass or insulin gene transcription, as it is selective for certain stimuli (17,18).

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HNF, hepatocyte nuclear factor; MODY, maturity-onset diabetes of the young.

TABLE 1
Description of six MODY genes

Locus	Human gene	Alias	Protein family	Function
MODY1	<i>HNF4A</i>	HNF4 α , NR2A1	Nuclear receptor	Transcriptional regulation
MODY2	<i>GCK</i>	Glucokinase, hexokinase IV	Hexokinase	Glycolysis, glucose sensing
MODY3	<i>TCF1</i>	HNF1 α , HNF1	Atypical homeodomain	Transcriptional regulation
MODY4	<i>IPF1</i>	IDX1, PDX1, STF1, GSF	Homeodomain	Transcriptional regulation
MODY5	<i>TCF2</i>	HNF1 β , vHNF1	Atypical homeodomain	Transcriptional regulation
MODY6	<i>NEUROD1</i>	NeuroD, Beta2	Basic helix-loop-helix	Transcriptional regulation

Studies in mice with homozygous HNF-1 α null mutations or expressing a dominant-negative inhibitor of HNF-1 have been conceptually consistent with the human MODY3 findings (19–25). *Hnf1 α ^{-/-}* mice do not exhibit a conspicuous developmental block, and although they have a small pancreas (and hence reduced β -cell mass), it is proportional to their small body weight (21,23). Nonetheless, minor derangements in the organization of pancreatic cells and altered adaptive regenerative capacity of β -cells likely exist (23). Insulin mRNA abundance has been shown to be either unaltered or moderately decreased in the case of the *ins1* gene (22–24). Analogous to the MODY3 findings, HNF-1 α -deficient islet cells display severely blunted secretory responses to glucose but retain the ability to respond to depolarization induced by KCl (19,23,25). The defective glucose response results in part from a glycolytic block proximal to enolase (19), but no single target gene is likely to be incriminated as a cause, given that numerous genes involved in stimulus secretion-coupling are under-expressed in *hnf1 α* -deficient islet cells (22,24,26). There is also no secretory response to arginine (20,23), perhaps because of decreased expression of the basic amino acid transporter BAT (24). Dominant-negative inhibition studies have additionally demonstrated effects on mitochondrial function (25). In the aggregate, it appears that a major function of HNF-1 α is to ensure that the machinery that is required for β -cells to respond to glucose and other stimuli is in place.

One apparent difference between HNF-1 α -null mutant mice and humans is the lack of a clear phenotype in heterozygous mice up to age 16 weeks (23,27). This discrepancy clearly needs to be assessed in greater detail, as there are several potential reasons to account for it. One is that diabetes in heterozygous humans is not present until 10–40 years after birth (13,15), which does not necessarily equate to 6–16 weeks in the life of a mouse. Another factor is the likely existence of epistatic genetic modifiers that could modulate penetrance in diverse genetic backgrounds. In keeping with this notion, heterozygosity at the *hnf1 α* locus in mice was recently shown to have striking effects on insulin secretion in a *pdx1^{+/-}* background (27). Interestingly, very late or nonpenetrance has been described in MODY3 mutation carriers, indicating not only inter- but also intraspecies phenotypic variability (8,15).

There is much less information on the role of HNF-4 α in β -cells. As occurs with HNF-1 α , young *hnf4 α ^{+/-}* mice do not exhibit glucose intolerance (27), whereas germ-line homozygous null mutants do not develop beyond gastrulation (28). Conditional Cre-loxP-based inactivation studies have shown that HNF-4 α has critical roles for numerous genes in adult hepatocytes (29), but analogous

experiments have yet to be reported in pancreatic cells. However, the β -cell phenotype in MODY1 is extremely similar to that in MODY3 (9,10). Furthermore, dominant-negative inhibition of either HNF-1 α or HNF-4 α in INS-1 β -cells leads to reduced expression of a remarkably similar set of genes (25,30). This suggests that HNF-1 α and HNF-4 α control a common genetic program in β -cells, and it is already quite clear that this program is required for the correct function of differentiated β -cells.

The function of HNF-1 α is fully dependent on cell-type context. Although homozygous HNF-1 α mutations have not been reported in humans, we know that *hnf1 α ^{-/-}* mice exhibit a severe pleiotropic phenotype in addition to diabetes (21,23,24,31–33). This results from widespread gene expression defects in liver, kidney, and other organs, leading to multiple derangements such as abnormal bile acid and cholesterol metabolism, phenylketonuria, fatty liver, growth impairment, and tubular glycosuria (21,23,24, 31–33). This picture contrasts completely with that seen in heterozygous HNF-1 α and HNF-4 α humans, who essentially present with β -cell dysfunction and diabetes (6,8–10,16). Isolated target gene defects in other tissues have been identified, such as decreased production of apolipoproteins AII and CII in MODY1 and an altered renal glucose reabsorption threshold in MODY3, but these are comparably subtle (32,34).

The tissue specificity of heterozygous mutations cannot be ascribed to a classical dominant-negative mechanism occurring solely in β -cells. A large number of human HNF-1 α and HNF-4 α mutations have been carefully analyzed, and although in some instances dominant-negative effects have been identified by overexpressing mutant proteins, there is compelling evidence in many other cases (such as in promoter mutations) that the mutation simply causes loss of function (14,35–37). Furthermore, the phenotype does not appear to be more severe in cases where a dominant-negative effect is observed (15). This suggests that HNF-1 α and HNF-4 α have β -cell-specific functions that are uniquely vulnerable to decreased gene dosage.

There is now more detailed information on some of the β -cell-specific functions of HNF-1 α . Numerous genes, including *glut2* and *pklr*, and several transcriptional regulators exhibit markedly decreased expression in *hnf1 α ^{-/-}* islets, but do not require HNF-1 α in other tissues such as liver (22,24,38). For many (though not all) genes, it is clear that the cell-specific requirements are not related to the hyperglycemic environment or other secondary derangements. For example, several such genes are abnormally expressed prenatally or shortly after birth, at a time when there is no significant hyperglycemia (22,24,38). Furthermore, HNF-1 α directly interacts with chromatin of targets such as *glut2* and *pklr* in mouse islets (22). Although some

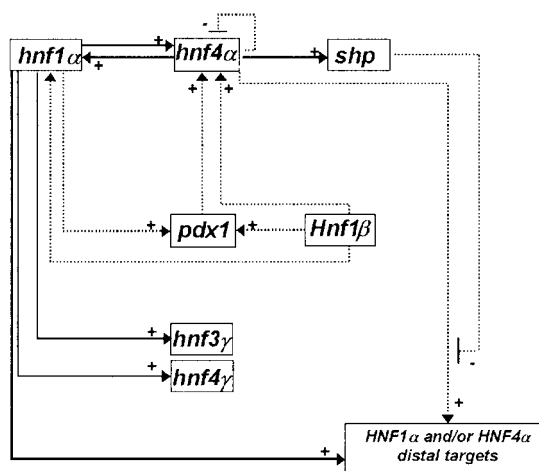


FIG. 1. Partial structure of a complex regulatory network in differentiated pancreatic cells. Solid lines represent interactions proven by genetic analysis, indicating an essential role in pancreatic cells, as well as by biochemical evidence, indicating that the interaction is direct. Dotted lines indicate interactions demonstrated by only one of these approaches. PDX-1 dependence on HNF-1 α was present in one of two genetic studies. Arrows with + signs indicate positive regulation and arrows with flat ends with negative signs indicate negative regulation. Note that SHP can exert posttranscriptional repression of several other nuclear receptors aside from HNF-4 α .

of the mechanisms involved in the activation of direct HNF-1 α targets are beginning to be unraveled (22,39,40), it is still unclear why for some genes HNF-1 α is required for activation in certain cell types but not in others.

HNF-1 α occupies a critical position in cell-specific transcriptional regulator networks. The existence of different HNF-1 α -dependent regulatory networks in diverse cell types could be one of several nonexclusive mechanisms that account for the cell-specific roles of HNF-1 α . The participation of HNF-1 α in transcriptional networks is well documented. The analysis of null mutant embryoid bodies has shown that HNF-3 β and HNF-3 α exert positive and negative control on HNF-4 α , respectively (41). HNF-4 α is required for normal transcription of HNF-1 α in hepatocytes and endoderm-like cells (37,41–43), whereas HNF-1 α controls downstream hepatic transcriptional activators, such as FXR (44). This complex network manages a vast array of targets encoding proteins involved in highly specialized liver functions (21,31,33,44).

In pancreatic islets, HNF-1 α is integrated in a network that has an entirely different architecture. Several mRNAs encoding transcriptional regulators, including HNF-4 α , HNF-4 γ , or HNF-3 γ , are dependent on HNF-1 α in pancreatic cells specifically (24,38) but are expressed normally in the liver or kidney of mice lacking HNF-1 α (24,38). Interestingly, HNF-1 α is also dispensable for the expression of these targets in pancreatic epithelial precursor cells, and only becomes essential shortly after differentiated pancreatic cells arise (38).

The existing data indicate that the structure of the pancreatic network is complex (Fig. 1) (24,30,38,45). It is nonlinear, with cross- and autoregulatory interactions (24,27,38). Some distal targets (such as *pklr* or *shp*) are connected with HNF-1 α indirectly through subsidiary activators or by both direct and indirect mechanisms (24,38,46). Most likely, any attempts to decipher the logic of this entire network design at this time would be based on a keyhole vision of a much more vast network.

The evidence for a cross-regulatory loop between HNF-1 α and HNF-4 α in pancreatic cells. It is striking that despite the complexity of this network, it is already possible to isolate a very simple substructure that exhibits a highly predictable behavior in different model systems. Thus, HNF-1 α and HNF-4 α have been shown to establish interdependence through direct cross-regulatory interactions in pancreatic cells (24,30,35,38) (Fig. 1).

Proof for HNF-1 α dependence on HNF-4 α comes from several HNF-4 α -deficient models that have shown that this gene is needed for normal HNF-1 α transcription in embryonic visceral endoderm and hepatocytes (37,42,43). This control is exerted through the interaction of HNF-4 α with a DR1 direct repeat *cis* element in the HNF-1 α promoter (35,47). Strongly suggestive evidence that this interaction also takes place in β -cells comes from two sources. First, dominant-negative inhibition of HNF-4 α in INS-1 β -cells results in severely decreased formation of HNF-1 α DNA-binding complexes and mRNA (30). Second, a loss of function mutation in the HNF-1 α promoter DR1 element is linked to MODY in a large pedigree (35). This suggests that HNF-4 α control of HNF-1 α occurs at some point in pancreatic development, and, importantly, that this interaction is essential for pancreatic β -cell function. Although a direct genetic analysis of the requirement for HNF-4 α to express HNF-1 α in both precursor and differentiated pancreatic cells is still needed, the available evidence indicates that HNF-4 α controls the expression of HNF-1 α in multiple cell types, including embryonic endoderm, liver, and pancreatic cells (30,35,37,41–43,47).

In sharp contrast, studies using *hnf1α*^{-/-} mice have shown that HNF-1 α control of HNF-4 α is clearly restricted to differentiated pancreatic cells (but not precursor cells), and in part to intestinal cells (24,38). Interestingly, HNF-4 α transcription in pancreatic cells is driven almost exclusively by an alternate promoter known as P2 (38,45). Chromatin immunoprecipitations have shown that HNF-1 α directly interacts with the P2 promoter in islets (38). HNF-1 α also transactivates P2 in transient transfection assays (45). Because another promoter (P1) is the predominant transcription initiation site in adult hepatocytes and kidney, HNF-4 α mRNA in *hnf1α*^{-/-} mice is not grossly deranged in these tissues (24,31,38). Thus, HNF-1 α is dependent on HNF-4 α in multiple tissues, but in pancreatic cells, a cross-regulatory loop between these two genes is formed (Fig. 2A).

The cross-regulatory circuit as a molecular memory device. Once insulin-producing cells arise, mechanisms need to be in place to maintain their phenotypic properties over time and throughout consecutive rounds of cell division. Cross-regulation between HNF-1 α and HNF-4 α is switched on as pancreatic cells are provided the signals to differentiate (Fig. 2B) (38). Once it is installed, this type of circuit configuration can provide a stable mechanism of gene expression, as physiological perturbations of the activity of one of the two genes can be reset back to the equilibrium state by the opposite gene (48,49). This can serve to self-perpetuate the activity of both genes and their targets in differentiated cells (Fig. 2B). The notion that a transient instructive signal is sufficient to lock a genetic program that maintains differentiated functions makes

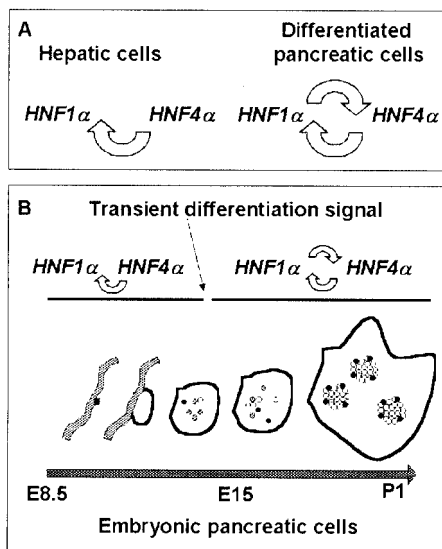


FIG. 2. A: Schematic representation of essential regulatory interactions between HNF-1 α and HNF-4 α in pancreatic and hepatic cells. **B:** Interdependence between HNF-1 α and HNF-4 α is established as pancreatic cells differentiate during embryogenesis. Cross-regulation can provide a mechanism to maintain stable expression over time, even if the initial instructive signal is short lived. Regulation of HNF-1 α by HNF-4 α in precursor pancreatic cells is conjectured based on findings in all cell types where it has been examined.

sense in a cell type that has little plasticity to undergo alternate fates.

A circuit with two stable states. Although interdependent activity represents the default stable state of this loop in differentiated pancreatic cells, under extreme conditions it could adopt an alternate equally stable state (Fig. 3A). If the function of one of the two genes is sufficiently perturbed so that the activity of the second gene is severely impaired, this can again inhibit the first gene and so on until the two genes reach a lower activity state. This

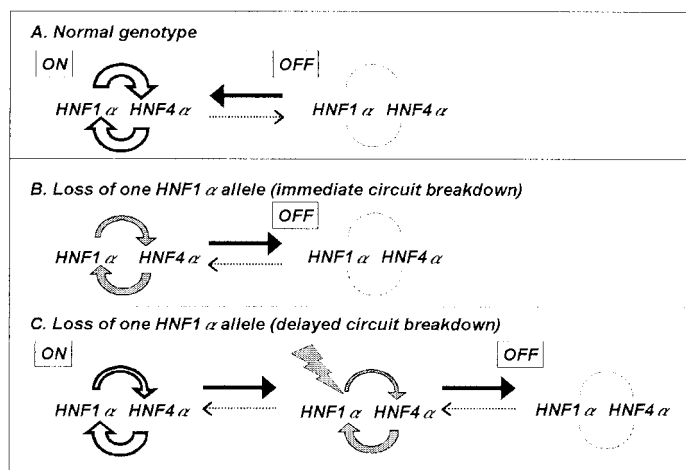


FIG. 3. Switch positions of the pancreatic HNF-1 α /HNF-4 α circuit. A: In the physiological stable ON state, the circuit configuration prevents inactivation. **B:** Loss of one allele (HNF-1 α in this case) results in circuit inactivation if 50% of HNF-1 α gene product is insufficient to maintain HNF-4 α gene activity at the level required to in turn maintain the activity of the normal HNF-1 α allele. **C:** Loss of one HNF-1 α allele is sufficient to maintain near-normal HNF-4 α activity. Haploinsufficiency increases the probability that a transient stochastic or deterministic fluctuation of HNF-1 α expression falls below a threshold level required for transition to the OFF state. Decreased gene dosage also increases the stability of the OFF state.

alternate equilibrium state is defined as the state in which the activity of each gene is that which takes place in the absence of the opposite gene. As discussed earlier, loss of function studies have indicated that for both genes this basal state represents a substantial reduction in gene expression. Intermediate states are unlikely, as insufficiently severe perturbations are reset to the original stable state. As in any switch mechanism, changing between ON and OFF positions requires a transient perturbation that exceeds a threshold level, but once the new position is reached it is stable, even if the triggering effect disappears. This type of bistable behavior has long been recognized as a property of positive cross-regulatory feedback circuits (48–51), although it requires experimental *in vivo* demonstration in this precise model. Simulated circuits very much like the HNF-1 α /HNF-4 α loop have been previously shown to exhibit bistability (48,49). Experimental verification of bistability has been attained in a synthetic single-gene, positive autoregulatory loop (52).

Are all four HNF-1 α and HNF-4 α alleles needed to keep the switch turned on? A bistable circuit configuration can have important implications for our understanding of the consequences of HNF-4 α and HNF-1 α haploinsufficiency. The loss of one functional allele can increase the probability that the opposite gene is inhibited sufficiently to trigger the transition to an OFF state (Fig. 3B). The net result of the loss of one allele in such a model is the reduction of activity of all four HNF-1 α or HNF-4 α alleles in pancreatic cells. Thus, an incomplete primary defect in any one of the two genes can lead to a phenotype that approaches that of a double homozygous null mutant. This concept is consistent with the severity of β -cell dysfunction imparted by the loss of a single allele (9), with the notion that the β -cell phenotype is very similar in MODY1 and -3 (9,10), and that dominant-negative inhibition of either HNF-4 α or HNF-1 α in β -cells causes reduced expression of the same set of genes (30).

The cellular specificity of the switch mechanism matches that of the heterozygous phenotype. If disruption of the loop is instrumental in the haploinsufficient mechanism, a severe phenotype should occur only in the cells in which the circuit exists (Fig. 2A). In the absence of a loop, the predicted outcome in nonpancreatic cells is that there could be partially decreased concentration of functional HNF-1 α in MODY3, or of HNF-4 α and perhaps HNF-1 α in MODY1, but not a severe phenotype resulting from impaired function of all four HNF-1 α and HNF-4 α alleles. This prediction closely fits the genetic findings. *Hnf1 α ^{-/-}* mouse studies have clearly shown that this gene is absolutely necessary for a vast array of critical genes in liver, gut, and kidney (21,23,24,31,32), whereas heterozygosity results in a clinically significant and severe phenotype only in pancreatic islet cells (6,8,9,16,27). Furthermore, although the heterozygous HNF-1 α and HNF-4 α phenotypes are very similar in β -cells, the mild liver and kidney defects reported in MODY1 and MODY3 are specific for each gene (32,34).

Delayed onset of the phenotype: When does circuit breakdown take place? Although MODY1 and -3 are caused by germ-line mutations, the diabetic phenotype does not appear until 10–40 years after birth (13,15). On the other hand, we know from the *hnf1 α ^{-/-}* mouse studies

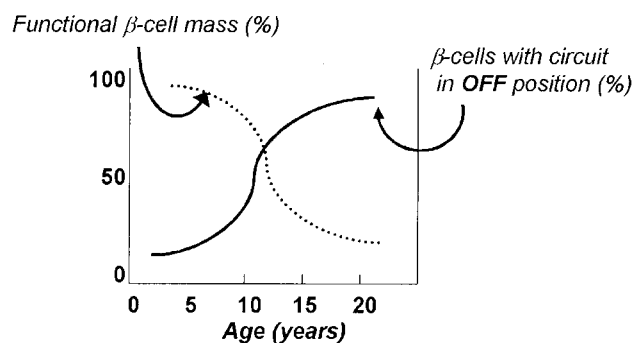


FIG. 4. Model for the delayed onset of the diabetic phenotype. Following the mechanism described in Fig. 3C, the time-dependent accumulation of β -cells that are switched to the stable OFF position eventually results in a mass of correctly functioning β -cells that is insufficient to maintain glucose homeostasis. A nonlinear relationship is depicted to accommodate the proposed interplay between extrinsic and stochastic factors, as described in the text.

that interdependence is installed soon after differentiated pancreatic cells appear during embryogenesis (Fig. 2B) (38). If the phenotype is dependent on the loop mechanism, why does it take so many years to develop?

Because HNF-4 α is not dependent on HNF-1 α in precursor cells (and hence no loop exists), the circuit is expected to be in the default ON state as insulin cells first appear in heterozygous mutation carriers. The question thus becomes, When does circuit inactivation take place? It could occur quickly in most cells soon after interdependence is established in embryonic insulin-producing cells (Fig. 3B). However, the fact that the phenotype does not appear until many years later makes it necessary to consider the alternate possibility that inactivation may be an inefficient process.

Most likely, the loss of a single HNF-1 α or HNF-4 α allele does not immediately compromise circuit activity in differentiated cells. This is clearly the case in mice, as young *hnf1 α ^{+/-}* animals express HNF-4 α mRNA at nearly normal levels in pancreatic cells (S. Boj and J.F., unpublished observations). However, transcription in a single cell is an inherently noisy process (53–56); it undergoes fluctuations that are not solely environmentally determined, but also stochastic (53,57). The existence of 50% of the normal gene product can critically increase the probability that an extreme fluctuation at any moment in the lifetime of a cell surpasses a threshold required to trigger the transition from the ON-to-OFF state (Fig. 3C). Perhaps even more relevant than the average activator concentration is the notion that single- as opposed to two-allele expression is on its own expected to increase this likelihood, as it leaves no backup activity if transient inhibition occurs in the single functional allele. Cook et al. (57) used computer simulations to demonstrate the increased probability that stochastic events lead to transient inactivity of a gene in a single- versus two-allele system, even under conditions in which average gene product concentration is held constant in both systems.

If the HNF-1 α /HNF-4 α circuit is bistable, a severe but transient inhibition can give rise to a permanent lock in the OFF state. The stability of this state is predicted to be reinforced by decreased gene dosage, as it should compromise circuit reactivation (Fig. 3). As more individual cells undergo the ON-to-OFF transition over the course of

years, a threshold of β -cells that possess an inadequate state of terminal differentiation is eventually achieved, giving rise to diabetes (Fig. 4). The average age at diagnosis could thus represent a function of the probability that the circuit is locked in an OFF state in a sufficient number of β -cells.

The notion that time, rather than solely age or biological maturation, is instrumental in enabling low-probability stochastic events to take place could underlie the lack of phenotype in heterozygous mice aged 6–16 weeks (23,27), in contrast to heterozygous humans aged 10–40 years (13,15). However, other factors aside from time are expected to regulate this process. The fact that penetrance is very rare before age 10 years in MODY3, and then increases progressively (13,15), may be attributable to age-related factors capable of increasing circuit instability, as opposed to an exclusive stochastic mechanism. On the other hand, penetrance variability, both within humans and between species, may result from genetic modifiers of circuit stability.

Interestingly, HNF-4 α mRNA levels are compensated in young heterozygous *+/-* mice, suggesting a negative autoregulatory loop (Fig. 1) (27). This effect is expected to counteract the ON-to-OFF transition. However, once the OFF state is attained, negative autoregulation can also counteract reactivation, and thus consolidate the stability of the inactive state.

The model postulated here is consistent with other parallel or consecutive pathogenic mechanisms. For example, circuit inactivation could take place long before the phenotype appears by limiting an appropriate cell growth or secretory adaptive response to demands imposed later in life, or by allowing gradual accumulation of cellular defects. On the other hand, once metabolic derangements are initiated, these are expected to contribute to the completion of the process of β -cell failure (58). Furthermore, decreased expression of HNF-1 α or HNF-4 α could conceivably trigger dysregulation of private network targets that are very sensitive to activator concentrations, before the inactivation of the loop.

Are other MODY genes required to keep the switch turned on? In addition to HNF-1 α and HNF-4 α themselves, other regulators are expected to control the activity of these two genes in differentiated pancreatic cells. The bistable circuit model predicts that any circumstance that imparts a severe inhibition of the loop below a threshold level can trigger its extinction, resulting in a similar phenotype as a mutation of either HNF-1 α or HNF-4 α . Other MODY genes are attractive candidate sites for this type of mechanism. Strong support for such an interaction among MODY genes has been provided by a recent study indicating that IPF-1 (MODY4) may be a critical regulator of HNF-4 α (45). Thomas et al. (45) discovered a mutation in the pancreatic HNF-4 α promoter that segregates with diabetes in a large kindred, with a logarithm of odds score of 3.25. The mutation disrupts an IPF-1 (PDX-1) high affinity binding site that is essential for HNF-4 α promoter activity (45). This suggests that the MODY4 phenotype could be mediated in part by a defective function of the HNF-1 α /HNF-4 α circuit (45). HNF-1 β (MODY5), a paralog of HNF-1 α that shares closely related DNA binding and dimerization domains (3,59), could also

regulate this circuit. *Hnf1 β* ^{-/-} embryos die before pancreas organogenesis but fail to express HNF-1 α in endoderm cells (60). HNF-1 β also interacts with the HNF-4 α P2 promoter in transient transfections and in vitro binding assays (45). Furthermore, HNF-1 β may act indirectly through IPF-1/PDX-1, as zebra fish HNF-1 β mutants fail to express PDX-1 in the endodermal segment destined to form the pancreatic bud (61).

An additional level of complexity lies in the observation by Shih et al. (24) of decreased expression of PDX-1 in pancreatic cells of *hnf1 α* ^{-/-} mice. A meaningful reduction of PDX-1 has not been observed in our own studies with embryonic and early postnatal pancreatic tissues from *hnf1 α* ^{-/-} mice (22,38) or in the dominant-negative inhibition cell line experiments (25). However, the PDX-1 5' flanking region contains an evolutionary conserved HNF-1 site (62,63), and this could conceivably be variably linked to HNF-1 α or HNF-1 β dependence in diverse genetic or environmental background settings. Thus, PDX-1 could lie both upstream and downstream of the HNF-1 α /HNF-4 α circuit, forming a separate regulatory loop. This notion is consistent with the existence of epistatic interactions between *pdx1* and *hnf1 α* loci in mice (27).

A framework to study the mechanism of haploinsufficiency of genetic network components. The mechanism of haploinsufficiency in human genetic diseases is greatly overrepresented among transcriptional regulator genes as compared with other functional categories (64–68). Aside from specific settings in which loss of heterozygosity and single allele expression have been described, the mechanism is generally based on the requirement of a critically narrow range of activator concentrations (64,66–68). Decreased gene dosage could lead to altered stoichiometry of multimeric regulatory complexes (68) or failure to establish cooperative interactions with other activators (69). The mechanism postulated here, for the first time to our knowledge, is that the consequences of decreased dosage of a single regulator gene can be greatly amplified in the face of a self-sustaining feedback circuit. Furthermore, it is proposed that in this setting, transient stochastic or extrinsically determined inhibitory events can serve as triggers for permanent network inactivation. Haploinsufficiency is postulated to increase both the likelihood of circuit inactivation and the stability of the inactive state. This notion broadens the implications of a study discussed earlier, whereby simulations were used to show that in the presence of a heterozygous loss of function mutation of any given gene, stochastic deactivation of the functional allele may intermittently mimic the homozygous null state (57). As cross-regulatory circuits between transcriptional regulators are extremely common (70,71), this raises the intriguing possibility that autocatalytic circuit breakdown could be involved in other haploinsufficient defects.

The model that is presented here is testable and requires experimental analysis in a β -cell context. For example, it is necessary to determine that extreme parameter conditions of the circuit, such as a very slow activation/inactivation rate, do not render extinction as the result of a transient event too unlikely. It is also important to know how the interplay with other network components affects the properties of the circuit. Nonetheless, the model rests on two firm premises. First, there is genetic evidence for pancre-

atic-restricted interdependence of HNF-1 α and HNF-4 α , which holds true irrespective of other mechanisms regulating these two genes (24,30,35,37,38,42,43,45,47). Second, the concept that positive cross-regulatory feedback circuits are capable of exhibiting multistability is well established (48–52). The model that emerges does a reasonably good job in explaining the established clinical features of MODY.

Such a framework to explain the haploinsufficient mechanism in MODY extends previous models that did not accommodate the existence of a feedback circuit, but did postulate that loss of one HNF-1 α allele results in insufficient activator concentrations to elicit normal target gene responses specifically in islets (36). Another model that did not incorporate this loop structure already postulated loss of function of both HNF-1 α alleles because of somatic loss of the wild-type allele in a subset of cells hypothesized to possess a growth advantage (14).

Final remarks. The obligate task of HNF-1 α and HNF-4 α in β -cells appears to be largely dependent on their position within a network and not solely on the linear activation of a set of critical distal target genes (24,38,45). Because one of the recognized attributes of complex networks is robustness to inactivation of random components (72), it is remarkable that the HNF-1 α /HNF-4 α subcircuit is unusually vulnerable. This vulnerability is characteristic of highly interconnected nodes within scale-free complex networks (72). An implication of such a hub position, together with the proposed bistable properties of the subcircuit, is that inhibition (perhaps even if transient) of HNF-1 α or HNF-4 α function could result in permanent damage to the differentiated state of β -cells. Critical regulatory mechanisms of this subcircuit may thus represent an attractive site for identifying new pathogenic mechanisms involved in the progression to β -cell dysfunction. Furthermore, an in-depth understanding of the design of this network may allow its manipulation and thus provide keys to build competent artificial insulin-producing cells.

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REFERENCES

1. Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ, Fajans SS, Signorini S, Stoffel M, Bell GI: Mutations in the hepatocyte nuclear factor-4 α gene in maturity-onset diabetes of the young (MODY1). *Nature* 384:458–460, 1996
2. Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M, Southam L, Cox RD, Lathrop GM, Boriraj VV, Chen X, Cox NJ, Oda Y, Yano H, Le Beau MM, Yamada S, Nishigori H, Takeda J, Fajans SS, Hattersley AT, Iwasaki N, Hansen T, Pedersen O, Polonsky KS, Bell GI: Mutations in the

- hepatocyte nuclear factor-1alpha gene in maturity-onset diabetes of the young (MODY3). *Nature* 384:455–458, 1996
3. Horikawa Y, Iwasaki N, Hara M, Furuta H, Hinokio Y, Cockburn BN, Lindner T, Yamagata K, Ogata M, Tomonaga O, Kuroki H, Kasahara T, Iwamoto Y, Bell GI: Mutation in hepatocyte nuclear factor-1 beta gene (TCF2) associated with MODY. *Nat Genet* 17:384–385, 1997
 4. Malecki MT, Jhala US, Antonellis A, Fields L, Doria A, Orban T, Saad M, Warram JH, Montminy M, Krolewski AS: Mutations in NEUROD1 are associated with the development of type 2 diabetes mellitus. *Nat Genet* 23:323–328, 1999
 5. Stoffers DA, Ferrer J, Clarke WL, Habener JF: Early-onset type-II diabetes mellitus (MODY4) linked to IPF1. *Nat Genet* 17:138–139, 1997
 6. Vaxillaire M, Boccio V, Philippi A, Vigouroux C, Terwilliger J, Passa P, Beckmann JS, Velho G, Lathrop GM, Froguel P: A gene for maturity onset diabetes of the young (MODY) maps to chromosome 12q. *Nat Genet* 9:418–423, 1995
 7. Tronche F, Yaniv M: HNF-1: a homeoprotein member of the hepatic transcription regulatory network. *Bioessays* 14:579–587, 1992
 8. Lehto M, Tuomi T, Mahtani MM, Widen E, Forsblom C, Sarelin L, Gullstrom M, Isomaa B, Lehtovirta M, Hyrkkö A, Kanninen T, Orho M, Manley S, Turner RC, Brettn T, Kirby A, Thomas J, Duyk G, Lander E, Taskinen MR, Groop L: Characterization of the MODY3 phenotype: early-onset diabetes caused by an insulin secretion defect. *J Clin Invest* 99:582–591, 1997
 9. Byrne MM, Sturis J, Menzel S, Yamagata K, Fajans SS, Dronsfield MJ, Bain SC, Hattersley AT, Velho G, Froguel P, Bell GI, Polonsky KS: Altered insulin secretory responses to glucose in diabetic and nondiabetic subjects with mutations in the diabetes susceptibility gene MODY3 on chromosome 12. *Diabetes* 45:1503–1510, 1996
 10. Byrne MM, Sturis J, Fajans SS, Ortiz FJ, Stoltz A, Stoffel M, Smith MJ, Bell GI, Halter JB, Polonsky KS: Altered insulin secretory responses to glucose in subjects with a mutation in the MODY1 gene on chromosome 20. *Diabetes* 44:699–704, 1995
 11. Fajans SS, Bell GI, Polonsky KS: Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med* 345:971–980, 2001
 12. Froguel P, Velho G: Molecular genetics of maturity-onset diabetes of the young. *Trends Endocrinol Metab* 10:142–146, 1999
 13. Owen K, Hattersley AT: Maturity-onset diabetes of the young: from clinical description to molecular genetic characterization. *Best Pract Res Clin Endocrinol Metab* 15:309–323, 2001
 14. Ryffel GU: Mutations in the human genes encoding the transcription factors of the hepatocyte nuclear factor (HNF-1) and HNF-4 families: functional and pathological consequences. *J Mol Endocrinol* 27:11–29, 2001
 15. Frayling TM, Evans JC, Bulman MP, Pearson E, Allen L, Owen K, Bingham C, Hannemann M, Shepherd M, Ellard S, Hattersley AT: Beta-cell genes and diabetes: molecular and clinical characterization of mutations in transcription factors. *Diabetes* 50 (Suppl. 1):S94–S100, 2001
 16. Surmely JF, Guenat E, Philippe J, Dussoix P, Schneiter P, Temler E, Vaxillaire M, Froguel P, Jequier E, Tappy L: Glucose utilization and production in patients with maturity-onset diabetes of the young caused by a mutation of the hepatocyte nuclear factor-1alpha gene. *Diabetes* 47:1459–1463, 1998
 17. Pearson ER, Liddell WG, Shepherd M, Corral RJ, Hattersley AT: Sensitivity to sulphonylureas in patients with hepatocyte nuclear factor-1 alpha gene mutations: evidence for pharmacogenetics in diabetes. *Diabet Med* 17:543–545, 2000
 18. Hansen T, Eiberg H, Rouard M, Vaxillaire M, Moller AM, Rasmussen SK, Fridberg M, Urhammer SA, Holst JJ, Almind K, Echwald SM, Hansen L, Bell GI, Pedersen O: Novel MODY3 mutations in the hepatocyte nuclear factor-1alpha gene: evidence for a hyperexcitability of pancreatic beta-cells to intravenous secretagogues in a glucose-tolerant carrier of a P447L mutation. *Diabetes* 46:726–730, 1997
 19. Dukes ID, Sreenan S, Roe MW, Levisetti M, Zhou YP, Ostrega D, Bell GI, Pontoglio M, Yaniv M, Philipson L, Polonsky KS: Defective pancreatic beta-cell glycolytic signaling in hepatocyte nuclear factor-1alpha-deficient mice. *J Biol Chem* 273:24457–24464, 1998
 20. Hagenfeldt-Johansson KA, Herrera PL, Wang H, Gjinovci A, Ishihara H, Wollheim CB: Beta-cell-targeted expression of a dominant-negative hepatocyte nuclear factor-1 alpha induces a maturity-onset diabetes of the young (MODY3)-like phenotype in transgenic mice. *Endocrinology* 142:5311–5320, 2001
 21. Lee YH, Sauer B, Gonzalez FJ: Laron dwarfism and non-insulin-dependent diabetes mellitus in the HNF-1alpha knockout mouse. *Mol Cell Biol* 18:3059–3068, 1998
 22. Parrizas M, Maestro MA, Fernandez S, Paniagua A, Casamitjana R, Gomis R, Rivera R, Ferrer J: Hepatic nuclear factor 1-alpha directs localized histone hyperacetylation of its tissue-specific transcriptional targets. *Mol Cell Biol* 21:3234–3243, 2001
 23. Pontoglio M, Sreenan S, Roe M, Pugh W, Ostrega D, Doyen A, Pick AJ, Baldwin A, Velho G, Froguel P, Levisetti M, Bonner-Weir S, Bell GI, Yaniv M, Polonsky KS: Defective insulin secretion in hepatocyte nuclear factor 1alpha-deficient mice. *J Clin Invest* 101:2215–2222, 1998
 24. Shih DQ, Sreenan S, Munoz KN, Philipson L, Pontoglio M, Yaniv M, Polonsky KS, Stoffel M: Loss of HNF-1alpha function in mice leads to abnormal expression of genes involved in pancreatic islet development and metabolism. *Diabetes* 50:2472–2480, 2001
 25. Wang H, Maechler P, Hagenfeldt KA, Wollheim CB: Dominant-negative suppression of HNF-1alpha function results in defective insulin gene transcription and impaired metabolism-secretion coupling in a pancreatic beta-cell line. *EMBO J* 17:6701–6713, 1998
 26. Wang H, Antinozzi PA, Hagenfeldt KA, Maechler P, Wollheim CB: Molecular targets of a human HNF-1alpha mutation responsible for pancreatic beta-cell dysfunction. *EMBO J* 19:4257–4264, 2000
 27. Shih DQ, Heimesaat M, Kuwajima S, Stein R, Wright CV, Stoffel M: Profound defects in pancreatic beta-cell function in mice with combined heterozygous mutations in Pdx-1, HNF-1alpha, and HNF-3beta. *Proc Natl Acad Sci U S A* 99:3818–3823, 2002
 28. Chen WS, Manova K, Weinstein DC, Duncan SA, Plump AS, Prezioso VR, Bachvarova RF, Darnell JE Jr: Disruption of the HNF-4 gene, expressed in visceral endoderm, leads to cell death in embryonic ectoderm and impaired gastrulation of mouse embryos. *Genes Dev* 8:2466–2477, 1994
 29. Hayhurst GP, Lee YH, Lambert G, Ward JM, Gonzalez FJ: Hepatocyte nuclear factor 4alpha (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis. *Mol Cell Biol* 21:1393–1403, 2001
 30. Wang H, Maechler P, Antinozzi PA, Hagenfeldt KA, Wollheim CB: Hepatocyte nuclear factor 4alpha regulates the expression of pancreatic beta-cell genes implicated in glucose metabolism and nutrient-induced insulin secretion. *J Biol Chem* 275:35953–35959, 2000
 31. Pontoglio M, Barra J, Hadchouel M, Doyen A, Kress C, Bach JP, Babinet C, Yaniv M: Hepatocyte nuclear factor 1 inactivation results in hepatic dysfunction, phenylketonuria, and renal Fanconi syndrome. *Cell* 84:575–585, 1996
 32. Pontoglio M, Prie D, Cheret C, Doyen A, Leroy C, Froguel P, Velho G, Yaniv M, Friedlander G: HNF-1alpha controls renal glucose reabsorption in mouse and man. *EMBO Rep* 1:359–365, 2000
 33. Akiyama TE, Ward JM, Gonzalez FJ: Regulation of the liver fatty acid-binding protein gene by hepatocyte nuclear factor 1alpha (HNF-1alpha): alterations in fatty acid homeostasis in HNF-1alpha-deficient mice. *J Biol Chem* 275:27117–27122, 2000
 34. Shih DQ, Danksy HM, Fleisher M, Assmann G, Fajans SS, Stoffel M: Genotype/phenotype relationships in HNF-1alpha/MODY1: haploinsufficiency is associated with reduced apolipoprotein (AII), apolipoprotein (CIII), lipoprotein(a), and triglyceride levels. *Diabetes* 49:832–837, 2000
 35. Gragnoli C, Lindner T, Cockburn BN, Kaisaki PJ, Gragnoli F, Marozzi G, Bell GI: Maturity-onset diabetes of the young due to a mutation in the hepatocyte nuclear factor-4 alpha binding site in the promoter of the hepatocyte nuclear factor-1 alpha gene. *Diabetes* 46:1648–1651, 1997
 36. Vaxillaire M, Abderrahmani A, Boutin P, Bailleul B, Froguel P, Yaniv M, Pontoglio M: Anatomy of a homeoprotein revealed by the analysis of human MODY3 mutations. *J Biol Chem* 274:35639–35646, 1999
 37. Stoffel M, Duncan SA: The maturity-onset diabetes of the young (MODY1) transcription factor HNF-4alpha regulates expression of genes required for glucose transport and metabolism. *Proc Natl Acad Sci U S A* 94:13209–13214, 1997
 38. Boj SF, Parizas M, Maestro MA, Ferrer J: A transcription factor regulatory circuit in differentiated pancreatic cells. *Proc Natl Acad Sci U S A* 98:14481–14486, 2001
 39. Pontoglio M, Faust DM, Doyen A, Yaniv M, Weiss MC: Hepatocyte nuclear factor 1alpha gene inactivation impairs chromatin remodeling and demethylation of the phenylalanine hydroxylase gene. *Mol Cell Biol* 17:4948–4956, 1997
 40. Soutoglou E, Viollet B, Vaxillaire M, Yaniv M, Pontoglio M, Talianidis I: Transcription factor-dependent regulation of CBP and P/CAF histone acetyltransferase activity. *EMBO J* 20:1984–1992, 2001
 41. Duncan SA, Navas MA, Dufort D, Rossant J, Stoffel M: Regulation of a transcription factor network required for differentiation and metabolism. *Science* 281:692–695, 1998
 42. Kuo CJ, Conley PB, Chen L, Sladek FM, Darnell JEJ, Crabtree GR: A transcriptional hierarchy involved in mammalian cell-type specification. *Nature* 355:457–461, 1992

43. Li J, Ning G, Duncan SA: Mammalian hepatocyte differentiation requires the transcription factor HNF-4 α . *Genes Dev* 14:464–474, 2000
44. Shih DQ, Bussen M, Sehayek E, Ananthanarayanan M, Shneider BL, Suchy FJ, Shefer S, Bollilini JS, Gonzalez FJ, Breslow JL, Stoffel M: Hepatocyte nuclear factor-1 α is an essential regulator of bile acid and plasma cholesterol metabolism. *Nat Genet* 27:375–382, 2001
45. Thomas H, Jaschko K, Bulman M, Frayling TM, Mitchell SM, Roosen S, Lingott-Frieg A, Tack CJ, Ellard S, Ryffel GU, Hattersley AT: A distant upstream promoter of the HNF-4 α gene connects the transcription factors involved in maturity-onset diabetes of the young. *Hum Mol Genet* 10:2089–2097, 2001
46. Miquerol L, Lopez S, Cartier N, Tulliez M, Raymondjean M, Kahn A: Expression of the L-type pyruvate kinase gene and the hepatocyte nuclear factor 4 transcription factor in exocrine and endocrine pancreas. *J Biol Chem* 269:8944–8951, 1994
47. Tian JM, Schibler U: Tissue-specific expression of the gene encoding hepatocyte nuclear factor 1 may involve hepatocyte nuclear factor 4. *Genes Dev* 5:2225–2234, 1991
48. Keller AD: Model genetic circuits encoding autoregulatory transcription factors. *J Theor Biol* 172:169–185, 1995
49. Thomas R, Thieffry D, Kaufman M: Dynamical behaviour of biological regulatory networks. I. Biological role of feedback loops and practical use of the concept of the loop-characteristic state. *Bull Math Biol* 57:247–276, 1995
50. Hasty J, McMillen D, Isaacs F, Collins JJ: Computational studies of gene regulatory networks: in numero molecular biology. *Nat Rev Genet* 2:268–279, 2001
51. Laurent M, Kellershohn N: Multistability: a major means of differentiation and evolution in biological systems. *Trends Biochem Sci* 24:418–422, 1999
52. Becskei A, Seraphin B, Serrano L: Positive feedback in eukaryotic gene networks: cell differentiation by graded to binary response conversion. *EMBO J* 20:2528–2535, 2001
53. McAdams HH, Arkin A: Stochastic mechanisms in gene expression. *Proc Natl Acad Sci U S A* 94:814–819, 1997
54. Dingemans MA, de Boer PA, Moorman AF, Charles R, Lamers WH: The expression of liver-specific genes within rat embryonic hepatocytes is a discontinuous process. *Differentiation* 56:153–162, 1994
55. Wijgerde M, Grosveld F, Fraser P: Transcription complex stability and chromatin dynamics in vivo. *Nature* 377:209–213, 1995
56. Ross IL, Browne CM, Hume DA: Transcription of individual genes in eukaryotic cells occurs randomly and infrequently. *Immunol Cell Biol* 72:177–185, 1994
57. Cook DL, Gerber AN, Tapscott SJ: Modeling stochastic gene expression: implications for haploinsufficiency. *Proc Natl Acad Sci U S A* 95:15641–15646, 1998
58. Weir GC, Laybutt DR, Kaneto H, Bonner-Weir S, Sharma A: Beta-cell adaptation and decompensation during the progression of diabetes. *Diabetes* 50 (Suppl. 1):S154–S159, 2001
59. Rey-Campos J, Chouard T, Yaniv M, Cereghini S: vHNF-1 is a homeoprotein that activates transcription and forms heterodimers with HNF-1. *EMBO J* 10:1445–1457, 1991
60. Barbacci E, Reber M, Ott MO, Breillat C, Huetz F, Cereghini S: Variant hepatocyte nuclear factor 1 is required for visceral endoderm specification. *Development* 126:4795–4805, 1999
61. Sun Z, Hopkins N: vHNF-1, the MODY5 and familial GCKD-associated gene, regulates regional specification of the zebra fish gut, pronephros, and hindbrain. *Genes Dev* 15:3217–3229, 2001
62. Ben Shushan E, Marshak S, Shoshkes M, Cerasi E, Melloul D: A pancreatic beta-cell-specific enhancer in the human PDX-1 gene is regulated by hepatocyte nuclear factor 3 β (HNF-3 β), HNF-1 α , and SPs transcription factors. *J Biol Chem* 276:17533–17540, 2001
63. Gerrish K, Cissell MA, Stein R: The role of hepatic nuclear factor 1 α and PDX-1 in transcriptional regulation of the pdx-1 gene. *J Biol Chem* 276:47775–47784, 2001
64. van Raamsdonk CD, Tilghman SM: Dosage requirement and allelic expression of PAX6 during lens placode formation. *Development* 127:5439–5448, 2000
65. Jimenez-Sanchez G, Childs B, Valle D: Human disease genes. *Nature* 409:853–855, 2001
66. Hermanns P, Lee B: Transcriptional dysregulation in skeletal malformation syndromes. *Am J Med Genet* 106:258–271, 2001
67. Seidman JG, Seidman C: Transcription factor haploinsufficiency: when half a loaf is not enough. *J Clin Invest* 109:451–455, 2002
68. Veitia RA: Exploring the etiology of haploinsufficiency. *Bioessays* 24:175–184, 2002
69. Bruneau BG, Nemer G, Schmitt JP, Charron F, Robitaille L, Caron S, Conner DA, Gessler M, Nemer M, Seidman CE, Seidman JG: A murine model of Holt-Oram syndrome defines roles of the T-box transcription factor Tbx5 in cardiogenesis and disease. *Cell* 106:709–721, 2001
70. Marquardt T, Pfaff SL: Cracking the transcriptional code for cell specification in the neural tube. *Cell* 106:651–654, 2001
71. Czerny T, Halder G, Kloter U, Souabni A, Gehring WJ, Buslinger M: twin of eyeless, a second Pax-6 gene of Drosophila, acts upstream of eyeless in the control of eye development. *Mol Cell* 3:297–307, 1999
72. Albert R, Jeong H, Barabasi AL: Error and attack tolerance of complex networks. *Nature* 406:378–382, 2000