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Hepatocyte Nuclear Factor 1, a Transcription Factor at the Crossroads of Glucose Homeostasis

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Abstract. Hepatocyte nuclear factor 1 (HNF1) is a transcription factor involved in the regulation of a large set of hepatic genes, including albumin, β-fibrinogen, and α1-antitrypsin. HNF1 is expressed in the liver, digestive tract, pancreas, and kidney. Mice lacking HNF1 exhibit hepatic, pancreatic, and renal dysfunctions. HNF1-deficient mice fail to express the hepatic phenylalanine hydroxylase gene, giving rise to hyperphenylalaninemia. Renal proximal tubular reabsorption of glucose, phosphate, arginine, and other metabolites is affected, producing severe renal glucosuria, phosphaturia, and amino aciduria. Homozygous mutant mice also exhibit a dramatic insulin secretion defect. This dysfunction resembles that exhibited by patients with maturity-onset diabetes mellitus of the young type 3, who carry mutations in the human HNF1 gene in the heterozygous state. These data show that HNF1 is a major regulator of glucose homeostasis, regulating the expression of genes that are expressed in the liver, kidney, and pancreas.

Cell differentiation is a rather elaborate program that is regulated largely at the transcriptional level. In this context, the concerted action of a limited number of transcription factors allows the selective activation of cell-specific genes. The study of transcription control regions of genes preferentially expressed in hepatocytes has led to the identification of several nuclear proteins that play important roles in liver-specific transcription. This set of transcription factors includes hepatocyte nuclear factor 1 (HNF1) and variant HNF1 (vHNF1) (also known as HNF1α and HNF1β or LF-B1 and LB-B3, respectively) (1–5), HNF3α, -β, and -γ (6), HNF4 (7), and HNF6 (8). HNF1 was first identified as a DNA-binding activity capable of interacting with the promoter of several hepatic genes, including albumin, α1-antitrypsin, and β-fibrinogen. HNF1 is a dimeric protein functionally composed of three domains, *i.e.*, an amino-terminal dimerization domain composed of 33 amino acids, a DNA-binding domain belonging to the homeobox family, and a carboxyl-terminal domain that is essential for transactivation of target promoters.

Among vertebrates, the closely related protein vHNF1 has also been characterized (4,5). HNF1 and vHNF1 share strong homologies in both the dimerization domain and the DNA-binding domain. These homologies enable the two proteins to form heterodimers and bind to the same DNA sequences. However, HNF1 seems to be a more potent transactivator than is vHNF1 in transient transfection assays. Another partner for HNF1, termed the dimerization cofactor for HNF1, has also been cloned (9). This protein binds to the amino-terminal domain of both HNF1 and vHNF1, stabilizing the homo- and heterodimers and somewhat increasing their transactivation potential. Surprisingly, the dimerization cofactor for HNF1 has also been shown to be involved in the enzymatic dehydration of pterin-4α-carbinolamine to dihydrobiopterin, a cofactor involved in the enzymatic hydroxylation of phenylalanine and tryptophan (10,11).

Studies have shown that HNF1 and vHNF1 are expressed in polarized epithelia of different organs, including the liver, digestive tract, pancreas, and kidney (12–15). The expression of vHNF1 overlaps with that of HNF1 with the exception of lung, where only vHNF1 is expressed. Conversely, vHNF1 is very weakly expressed in liver, where HNF1 constitutes >95% of the total HNF1-like protein. In other organs, HNF1 and vHNF1 are more or less equally expressed. In the kidney, HNF1 expression is confined to proximal tubules. In fact, immunofluorescence studies show that HNF1-positive nuclei are observed only in tubules that are also positive for villin, a marker of proximal tubules (Figure 1). In contrast, vHNF1 is also expressed in distal tubules (12–15).

A significant difference between HNF1 and vHNF1 is the onset of their expression during embryonic development. vHNF1 is expressed in the extraembryonic visceral endoderm, in the neural tube, and in the primitive gut at a developmental stage when HNF1 expression has not yet been turned on (16–18). In fact, HNF1 expression is activated only during organogenesis. Again, during liver and renal development, vHNF1 is expressed from the first stages of organogenesis, whereas HNF1 is turned on later, when differentiation is more advanced. For example, during kidney development, vHNF1 is already activated in the swelling ureteric bud and in the surrounding metanephric mesenchyme, whereas HNF1 becomes detectable during formation of the S-shaped structures (19). The differences between HNF1 and vHNF1 in their expression patterns and timing during development are also reflected in the outcomes of gene knockout experiments in mice. Although

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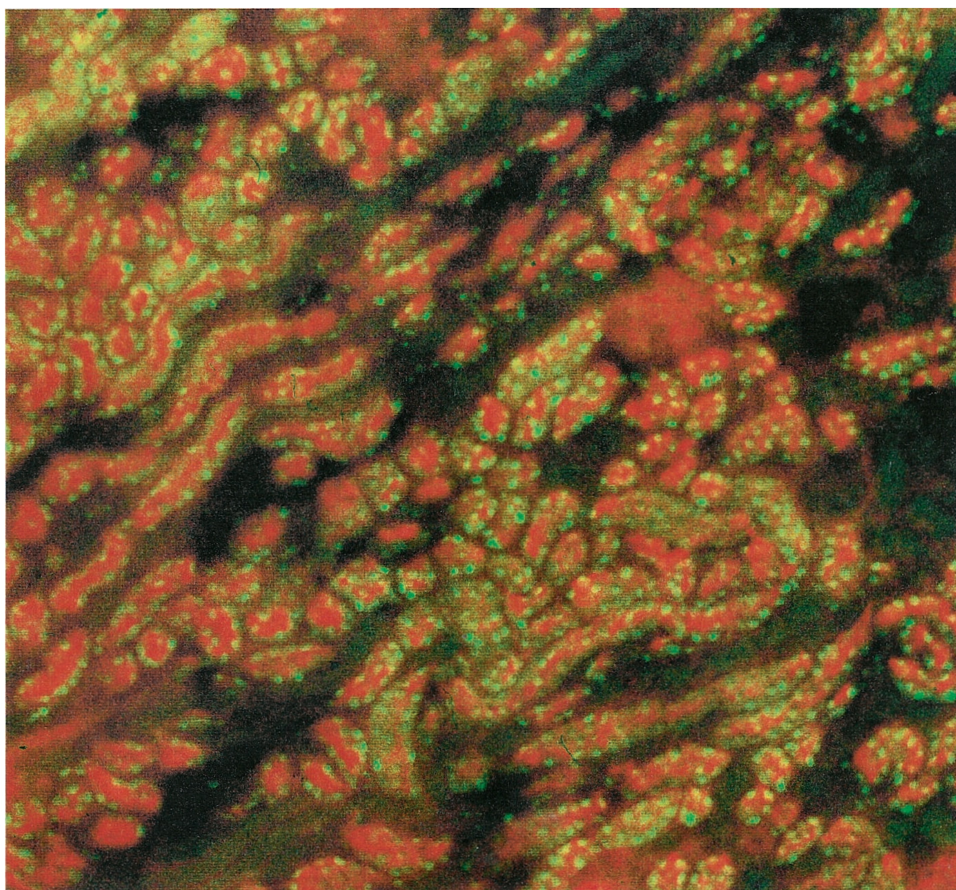

Figure 1. Localization of hepatocyte nuclear factor 1 (HNF1) in kidney. Cryosections from mouse kidneys were labeled by indirect immunofluorescence staining with antibodies against HNF1 (antibody TC284) (34) (green) and antibodies against villin (kindly provided by Silvie Robine, Institut Curie, CNRS UMR 144, Paris, France) (red).

vHNF1$^{-/-}$ mice embryos die at day 6.5 to 7.0 after conception, because of a defect in extraembryonic visceral endoderm differentiation (17,18), disruption of the mouse HNF1 gene leads to a complex set of postnatal dysfunctions that affect most of the organs in which HNF1 is expressed (13). HNF1$^{-/-}$ mice do not die as embryos but exhibit hepatic, pancreatic, and renal dysfunctions that significantly affect the growth and life spans of the mutant animals.

Liver Dysfunction

HNF1-deficient mice exhibit significant liver enlargement. Analysis of plasma biochemical parameters revealed extensive hypercholesterolemia and hyperphenylalaninemia. The catabolism of phenylalanine, an essential amino acid, includes an obligatory step involving the conversion of phenylalanine to tyrosine. This hydroxylation reaction is catalyzed by phenylalanine hydroxylase (PAH), an enzyme that is normally expressed in liver and to a lesser extent in kidney. Mutations in the human PAH gene can compromise this enzymatic activity and provoke the accumulation of phenylalanine in the plasma, giving rise to the phenylketonuric syndrome (20). Analysis of the expression of the murine PAH gene in HNF1$^{-/-}$ animals demonstrated that this gene was totally silent (13). This result was surprising, because all other known HNF1 targets, such as albumin, α1-antitrypsin, and β-fibrinogen, exhibited only moderate decreases (two- to fourfold) in their transcription rates. The corollary of these observations is that the lack of HNF1 results in selective and drastic inactivation of only a small subset of hepatic genes. The PAH gene, which is normally responsive to glucocorticoids and cAMP, cannot be induced by any hormonal treatment in the liver of HNF1-deficient animals (21). This lack of inducibility is probably attributable to the fact that the PAH gene in the hepatocytes of HNF1-deficient mice is characterized by an inactive chromatin structure in which several nuclease-hypersensitive sites, located in the transcription control regions of the PAH gene, have disappeared. These nuclease-sensitive sites contain several HNF1-binding sites and should play an important role in the transcriptional activation of the PAH gene. In addition to the poorly accessible chromatin status, PAH transcription control regions are characterized by hypermethylation of CpG residues in the DNA sequence. Therefore, the lack of HNF1 prevents remodeling and demethylation of the PAH locus. In this context, HNF1 seems to play an essential role by inducing chromatin remodeling events that render the transcription control region of the PAH gene accessible to the transcription machinery. It will be important to elucidate the mechanisms involved in the HNF1-dependent demethylation and chromatin remodeling processes.

In the kidney, where relatively high levels of vHNF1 persist in HNF1$^{-/-}$ mice, the normal low expression level of PAH is not affected. This suggests that vHNF1 might have taken over the role of HNF1 for this function in the kidney.

Kidney Dysfunction

HNF1-deficient mice exhibit drastic reductions in the proximal reabsorption of several metabolites. The vast majority of filtered glucose, as well as phosphate and some specific amino acids such as arginine, are abundantly lost in the urine, instead of being reabsorbed by the proximal tubules (13). The relative high concentrations of glucose and other metabolites induce osmotic diuresis, which causes the animals to become polyuric and polydipsic. The reabsorption of most of these metabolites is based on secondary active transport performed by Na^+-dependent cotransporters. The driving force for this transport is the electrochemical gradient of Na^+ across the luminal membrane of proximal tubular cells.

Phlorizine, which is a glucoside capable of inhibiting glucose reabsorption, is able to bind irreversibly to glucose cotransporters. The use of this inhibitor has demonstrated that brush border membranes of $HNF1^{-/-}$ animals exhibit large decreases in the number of phlorizine-binding sites (13). Glucose reabsorption is accomplished by two distinct cotransporters, termed sodium glucose transporter type 1 (SGLT1) and SGLT2 (22–25). SGLT2 is preferentially expressed in the initial portion (S1 and S2) of proximal tubules. There the concentration of glucose is still relatively high and SGLT2 is responsible for so-called high-capacity/low-affinity glucose transport. In the more distal part of the proximal tubule (S3), the glucose concentration is decreased and SGLT1, which is preferentially expressed in this segment, performs low-capacity/high-affinity transport. SGLT1 and SGLT2 have different glucose/Na^+ stoichiometries (1:2 and 1:1 for SGLT1 and SGLT2, respectively) (20). This enables SGLT1 to reabsorb glucose against the much stronger glucose gradient that is characteristic of the more distal part of proximal tubules.

Northern blot analysis of renal mRNA has demonstrated that SGLT1 is normally expressed, whereas transcription of the SGLT2 gene is severely affected, in the kidneys of HNF1-deficient animals (26). In addition, isolated tubules are characterized by a reduced capacity to reabsorb glucose, phosphate, and arginine but not alanine, an amino acid that is reabsorbed normally *in vivo* in $HNF1^{-/-}$ animals (unpublished results).

Pancreas Dysfunction

Mutations in the human HNF1 gene, in the heterozygous state, have been found to be responsible for a particular form of diabetes mellitus termed maturity-onset diabetes mellitus of the young type 3 (MODY3) (27). Affected patients are characterized by an insulin secretion defect that becomes apparent before 25 yr of age (28,29). The blood glucose levels of $HNF1^{+/-}$ mice are completely normal and those of HNF1-deficient mice are not particularly elevated; however, the severe renal glucosuria that affects mutant homozygous mice could have masked an insulin secretion defect. In fact, *in situ* glucose pancreas perfusion experiments demonstrated that pancreata from mutant mice are strongly affected in their capacity to secrete insulin in response to glucose (30). However, insulin is secreted when depolarizing solutions of KCl are perfused, indicating that the Langerhans islets of HNF1-deficient animals still produce insulin but their β-cells have a major defect in glucose-sensing. In addition, metabolic studies have demonstrated that the flux of glucose through glycolysis in islets from mutant mice is reduced, producing blunted increases in ATP levels in response to glucose exposure (31).

HNF1-deficient mice exhibit defective insulin secretion as well as a major defect in renal glucose reabsorption. The combination of these two dysfunctions establishes a novel dynamic equilibrium for glucose homeostasis. Renal glucosuria at least partially compensates for the defective insulin secretion.

MODY3 Mutations

MODY3 in human patients is inherited in a dominant manner, whereas the dysfunctions observed in $HNF1^{-/-}$ mice are transmitted as a recessive trait. In fact, $HNF1^{+/-}$ heterozygous mice do not exhibit any insulin secretion defect or glucose intolerance. This still-unresolved paradox is also observed for another form of diabetes mellitus. Mutations in the human HNF4 gene result in the MODY1 diabetic phenotype (32). Again, affected patients are heterozygous, whereas heterozygous mice carrying a mutation are perfectly normal (33). To date, >80 independent mutations have been identified in patients with MODY3. Analysis of these mutations has demonstrated that some of them render the protein very unstable and poorly expressed (because it is rapidly degraded) (34). Other mutations affect either DNA binding or transactivation potential. Finally, a subgroup of mutations exert a dominant-negative effect over the wild-type protein. However, patients with the latter type of mutation do not exhibit a more severe phenotype (34). In mice, it is possible that just one copy of either the HNF1 or HNF4 wild-type gene is sufficient to accomplish the functions exerted by the transcription factors. In contrast, in patients with MODY1 or MODY3, the presence of just one copy of HNF4 or HNF1 could lead to a haploid insufficiency in the pancreas in adulthood.

Conclusion

HNF1 plays an important role in controlling the postnatal functions of the liver, pancreas, and kidney. The phenotype of $HNF1^{-/-}$ mice has revealed that this transcription factor is crucial for the transcriptional activation of genes that play key roles in phenylalanine catabolism, pancreatic β-cell glucose-sensing, and renal proximal tubular reabsorption of glucose and several other metabolites. For these reasons, HNF1 can be considered a transcription factor at the crossroads of the regulation of glucose homeostasis.

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