Cell type-specific Transcriptional Activation and Suppression of the α1B Adrenergic Receptor Gene Middle Promoter by Nuclear Factor 1*

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Nuclear factor 1 (NF1) has been reported to be a transcriptional activator for some genes and a transcriptional silencer for others. Here we report that in Hep3B cells, cotransfection of NF1/L, NF1/Red1, or NF1/X with the α1B adrenergic receptor (α1BAR) gene middle (P2) promoter increases P2 activity to more or less the same degree, whereas in DDT1 MF-2 cells cotransfection of NF1/L or NF1/Red1 causes a small but statistically significant decrease in the P2 promoter activity, and NF1/X causes a greater, 70% inhibition. Further experiments using truncated NF1/X mutants indicate that NF1/X contains both positive and negative regulatory domains. The positive domain, located between amino acids 243 and 416, is active in Hep3B cells, whereas the negative domain, located between amino acids 416 and 505, is active in DDT1 cells, whereas the negative domain, located between amino acids 243 and 416, is active in DDT1 MF-2 cells. These functional domains are also capable of regulating transcription when isolated from their natural context and fused into the GAL4 binding domain. Furthermore, NF1 affinity purified from rat liver nuclear extracts copurified with a non-DNA binding protein, which can bind to the P2 promoter of the α1BAR gene via interacting with NF1. Taken together, these findings indicate that NF1/X contains both activation and suppression domains that may be recognized and modulated by cell type-specific cofactors. This may be one of the mechanisms whereby NF1 can activate or suppress the expression of different genes, and it may also underlie the tissue-specific regulation of the α1BAR gene.

The α1B adrenergic receptor (α1BAR) is a G-protein-coupled receptor that plays an important role in the acute control of cardiovascular homeostasis and metabolic processes in the liver and is also involved in promoting cell proliferation in various tissues (1). Expression of the α1BAR gene is regulated by hormonal and developmental factors in a tissue-specific manner, as exemplified in hypothyroidism, which increases the level of α1BAR mRNA in the rat heart but decreases it in the rat liver (2). In primary cultures of rat hepatocytes, high cell density prevents the decline in α1BAR expression observed at low cell densities (3), whereas in primary cultures of myocardicocytes, increasing cell density decreases α1BAR expression (4). As a first step toward understanding the molecular mechanisms responsible for such complex regulation, we cloned the rat α1BAR gene and identified the multiple promoters and cis-acting elements in its regulatory domain (5–7). In subsequent experiments we have found that the dominant P2 promoter interacts with multiple transcription factors including NF1,1 CP1, AP2, and CREB (8–10). Further data showed that NF1 and Sp1 are the major transcription factors involved in controlling the P2 promoter in liver and in DDT1 MF-2 smooth muscle cells, respectively (9).

NF1 represents a family of sequence-specific DNA binding proteins that bind to the TGGNNCC consensus sequence (11). NF1 has been reported to act as a transcriptional silencer for some genes, such as the genes encoding retinol-binding protein (12), 3-hydroxy-3-methylglutaryl-coenzyme A reductase (13), AP1 (14), growth hormone (15), mouse o21I collagen (16), von Willebrand factor (17), and peripherin (18), but it acts as a transcriptional activator for other genes, including the α-globin gene (19), human hepatitis B virus S gene (20), P53 gene (21), and the gene encoding myelin basic protein (22). The molecular basis for this duality remains unknown. Here we report that NF1 activates the transcription of the α1BAR gene in Hep3B cells and in primary rat hepatocytes but inhibits it in DDT1 MF-2 smooth muscle cells. To our knowledge, this is the first example for NF1 acting either as a positive or as a negative regulator for the same gene in different cells. The results further indicate that NF1/X contains both positive and negative regulatory domains, which remain functional when isolated from their natural context and which display cell type-specific activity. We also provided evidence for the presence in rat liver of a non-DNA binding protein interacting with NF1.

MATERIALS AND METHODS

Cell Culture—The DDT, MF-2 hamster smooth muscle cell line and the HepG2 human hepatocellular carcinoma cell line were obtained from the American Type Culture Collection (Rockville, MD) and cultured under conditions specified by the supplier. The primary culture of rat hepatocytes was described previously (8).

Construction of Plasmids—The pNF1/X, pNF1/Red1, and pNF1/L expression vectors were prepared as described previously (8). The deleted NF1 expression vectors are prepared by subcloning the cDNAs encoding a set of truncated NF1 proteins into pcDNA3 expression vectors. These cDNAs were generated by polymerase chain reaction amplification. The G4X, G4X416, and G4X243 fusion protein expression constructs were prepared by linking the NF1/X domains aa 139–505, 139–416, or 139–243 in frame into the pG4 vector. The pG4 vector expresses the GAL4 (aa 1–147) DNA binding domain (23). The reporter plasmid G4TK/CAT contains five GAL4 binding sites in the Tk promoter/CAT vector (23). All constructs were verified by restriction mapping and sequencing.

Transient Transfections and CAT Assays—Transient transfections and CAT assays were performed as described previously (6).

1 The abbreviations used are: NF1, nuclear factor 1; aa, amino acids; CAT, chloramphenicol acetyltransferase.
DNA Gel Mobility Shift Assay—DNA gel mobility shift assay was described previously (7). The double-stranded oligo II: 5'-CGA CCC GCG TGG CGC TGG CGT GCG GCC GCG CCT TGG CTC GAC CCG CAT TGC CCC CTA-5'; −543 to −484 from rat α1bAR gene 5'-flanking region) was used as a probe.

Partial Purification of NF1 Protein—Crude liver nuclear extract (1.1 g obtained from 1000 g of livers) was applied to a 200-ml DEAE-Sepharose column pre-equilibrated with buffer E (20 mM HEPES, pH 7.9, 15 mM MgCl2, 0.2 mM EDTA, 10% glycerol, 0.01% Nonidet P-40) containing 300 mM KCl. The flow-through containing the active fractions was diluted to 100 mM KCl with buffer E and loaded onto a 90-ml heparin-Sepharose CL-6B column equilibrated with buffer E containing 100 mM KCl. The column was eluted with buffer E containing 100–700 mM KCl increased in 50 mM steps, and fractions were analyzed by DNA gel mobility shift assay using 32P-labeled oligo II as a probe.

RESULTS

NF1 Is a Positive and Negative Transcriptional Regulator of the Rat α1bAR Gene P2 Promoter in Hep2 Cells and DDT1 MF-2 Cells, Respectively—Our previous data showed that NF1/L, NF1/Red1, and NF1/X were able to activate transcription via the P2 promoter to more or less the same degree in Hep3B cells as in primary cultured hepatocytes (8). We wondered whether these NF1 isoforms can also regulate the P2 promoter activity in DDT1 MF-2 smooth muscle cells. The P2/CAT construct was cotransfected into DDT1 MF-2 cells with the NF1/L, NF1/Red1, or NF1/X expression vectors. Unexpectedly, co-expression of NF1/L or NF1/Red1 caused small but statistically significant decreases in P2 promoter activity, whereas co-expression of NF1/X caused a greater, 70% inhibition (Fig. 1). These decreases were not due to variations in transfection efficiency, because the β-galactosidase vector was cotransfected as an internal control and similar β-galactosidase activities were obtained in all cotransfection experiments (data not shown).

We also cotransfected the mutated P2 m/CAT construct, which does not bind NF1, with the NF1/X expression vector into DDT1 MF-2 cells. The P2 m/CAT promoter activity was 30% higher than the activity of the wild type P2/CAT (data not shown), which is compatible with NF1 being a negative regulator of the transcription of the same gene, in a cell type-specific manner. Because NF1/X exerted the strongest inhibition and it was also a strong activator, we further studied the regulation of the activity of the P2 promoter by NF1/X to identify its transcriptional activator and repressor domains. We prepared two truncated NF1/X expression vectors, NF1/X416 and NF1/X243, which contain the NF1/X coding region aa 1–416 and aa 1–243, respectively. These truncated vectors were cotransfected with a P2 promoter/CAT construct into Hep3B or DDT1 MF-2 cells. After 60 h, the cells were harvested and CAT activities were measured. As seen in Fig. 1, in Hep3B cells cotransfection of 1 μg of NF1/X resulted in a 4.5-fold increase in P2 promoter activity. When 2 μg of the NF1/X vector was cotransfected in Hep3B cells, a greater, 13-fold increase in P2 promoter activity was observed, whereas co-transfection of the NF1/X416 or NF1/X243 vectors did not significantly affect the activity of the P2 promoter (Fig. 2). This suggests that the NF1/X region between aa 416 and 505 contains a transcriptional activator domain, which is active in Hep3B cells. Similar cotransfection in DDT1 MF-2 cells resulted in a 70% inhibition of P2 promoter activity by NF1/X and NF1/X416, whereas NF1/X243 was not inhibitory. This sug-
suggests that the NF1/X region between aa 243 and 416 contains a transcriptional repressor domain.

The Cell Type-specific Positive and Negative Regulatory Domains of NF1/X Are Functional When Isolated from Their Natural Context—To verify whether the above functional domains are capable of regulating transcription when isolated from their natural context, the NF1/X domains corresponding to aa 139–505, 139–416, or 139–243 (thus lacking the DNA-binding N-terminal domain of NF1/X) were linked in frame into the pG4 vector to produce the G4/X, G4/X416, or G4/X243 fusion protein expression constructs, respectively. The pG4 vector expresses the GAL4 (aa 1–147) DNA binding domain. These fusion protein constructs were cotransfected into Hep3B and DDT1 MF-2 cells with a reporter plasmid G4TK/CAT, which contains five GAL4 binding sites in the TK promoter/CAT vector. Similar cotransfection with pTK/CAT served as a negative control. This assay eliminates the interference caused by the possible binding of endogenous NF1 to the reporter constructs. As shown in Fig. 3, the G4/X construct stimulated 505 represents a reporter plasmid containing five GAL4-binding sites in the TK promoter/CAT constructs. TK represents a reporter TK promoter/CAT vector. 2 μg of the G4/X, G4/X416 or G4/X243 expression vectors were cotransfected into Hep3B or DDT1, MF-2 cells with 2 μg of a TKG4/CAT or TK/CAT construct. After 72 h, CAT activities were measured. CAT activity is expressed as a percentage of control, as established by using TKG4/CAT alone in Hep3B cells. Data are the means ± S.E. from four independent experiments.

NF1/X contains positive and negative regulatory domains that retain cell type-specific activity when isolated from their natural context. The transcriptional activities of different fusion proteins were examined in transient expression assays. TKG4 represents a reporter plasmid containing five GAL4-binding sites in the TK promoter/CAT constructs. TK represents a reporter TK promoter/CAT vector. 2 μg of the G4/X, G4/X416 or G4/X243 expression vectors were cotransfected into Hep3B or DDT1, MF-2 cells with 2 μg of a TKG4/CAT or TK/CAT construct. After 72 h, CAT activities were measured. CAT activity is expressed as a percentage of control, as established by using TKG4/CAT alone in Hep3B cells. Data are the means ± S.E. from four independent experiments.

Evidence for the Presence in Rat Hepatocytes of a Non-DNA Binding Protein Interacting with NF1—There is increasing evidence that NF1 can interact with a number of proteins, such as histone H3 (24), the Ski oncoprotein (25), ATF-2 (26), MEF3 (27), HNF3 (28), and some unidentified cofactors (29). We were wondering whether the cell type-specific positive and negative transcriptional regulation by NF1/X is due to the presence of cell type-specific cofactors that modulate NF1/X activity. As shown in previous studies (see Fig. 4A in Ref. 7 and Figs. 2A and 5A in Ref. 8), oligo II binds two major and one minor band. The results of competition experiments indicated that the minor band is CP1, and the two major bands contain the NF1 protein. During DNA affinity chromatography of NF1, we noticed that only the lower band of the major NF1 complex was recovered after the heparin-Sepharose step (0.55–0.65 M KCl fractions, twelfth through fourteenth lanes in Fig. 4A), the top band was lost. This could suggest that crude extracts contain a factor(s) that does not bind to oligo II directly but contacts NF1 through protein-protein interactions to form the top band in gel shift assays. To test this possibility, we performed a mixing experiment in which the fraction eluted by a 100-fold excess of unlabeled NF1 oligo (data not shown). The results suggest that proteins present in the 0.35 or 0.4 M KCl fraction do not bind the NF1 recognition sequence directly but interact through protein-protein contacts with the NF1-DNA complex.

**DISCUSSION**

In the present paper we provide the first published example of a transcription factor, NF1, acting as either a positive or a
negative regulator of the same gene in a cell-specific manner. NF1 activated the transcription of the α1βAR gene through its dominant P2 promoter in Hep3B cells and in primary rat hepatocytes but inhibited it in DDT, MF-2 smooth muscle cells. Further studies indicated that NF1/X contained both positive and negative regulatory domains. The positive domain, which is located between aa 416 and 505, is active in Hep3B cells, whereas the negative domain, located between aa 243 and 416, is active in DDT, MF-2 cells. These domains are also functional when isolated from their natural context. Furthermore, we provide evidence for the presence in rat liver of a non-DNA binding protein interacting with NF1. This suggests that the cell type-specific positive and negative transcriptional control by NF1 may be due to modulation of its activity by cell type-specific cofactors. These findings may also help us to understand the molecular basis for NF1 acting either as a transcriptional silencer or as an activator for different genes.

Transcriptional activation by NF1 is mediated by a proline-rich activator region in its C-terminal domain, which enhances transcription through direct interactions with TFIIB and requires a CTD-like sequence (SPTSPSY) (30–33). It has been also reported that transcriptional activation by NF1/CTF1 is mediated by a bipartite low-proline domain (34). The NF1/X protein does not contain a proline-rich region, and the present data demonstrates that its transcriptional activation domain is located between aa 416 and 505. Furthermore, this domain was functionally active in Hep3B but not in DDT, MF-2 cells. How this region activates gene transcription remains unknown.

The mechanisms by which NF1 can repress gene transcription also remain unknown. Several possibilities have been proposed: 1) In reverse-oriented transcription from the inverted terminal repeat of human type 5 adenovirus, NF1 is believed to exert its negative role by stoichiometrically interfering with the binding of a TATA box binding protein, therefore preventing transcription from that site (35). 2) A switch from activation to repression by NF1 might simply result from the differences in the linear organization of the surrounding target sequences (36). 3) Subtle variations in the nucleotide sequence of the NF1 binding site might represent another mechanism by which the cell can switch a particular NF1 isoform from a positive to a negative transcriptional regulator (37), as has been shown for other transcription factors, such as the glucocorticoid receptor, where the presence of an adenine at position 12 in the consensus glucocorticoid response element is the likely cause for a switch from activation to negative transcriptional regulation (37). 4) It has been also proposed that the C terminus of NF1 may be involved in protein-protein interactions (13), by analogy to a similar mechanism in the case of the negative regulator element of the peripherin gene (18). However, there is no published information on any specific domain of NF1 that might be involved in transcriptional repression. The present data demonstrate that NF1/X contains a transcriptional repression domain located between aa 243 and 416, which is active in DDT, MF-2 cells but not in Hep3B cells.

Why does NF1 act as a positive regulator of α1βAR gene transcription in Hep3B cells, but as a negative regulator in DDT, MF-2 cells? It is widely accepted that NF1 regulates transcription via its C-terminal region, which recognizes other components of the transcription machinery. There is increasing evidence to show that NF1 can interact with many proteins, such as histone H3 (24), the Ski oncoprotein (25), ATF-2 (26), MEFP3 (27), HNF3 (28), and some unidentified cofactors (29). Therefore, the cell type-specific positive and negative transcriptional regulation by NF1/X may be due to the presence of a positive cofactor(s) that binds NF1/X region aa 416 and 505 to activate gene transcription in Hep3B cells and due to the presence of a negative cofactor(s) that binds NF1/X region aa 243 and 416 to repress gene transcription. Indeed, the data from the affinity purification of NF1 suggested that liver nuclear extracts contain a non-DNA binding protein that may bind to NF1 and thus interacts indirectly with the P2 promoter of rat α1βAR gene. Whether HepG2 cells and DDT, MF-2 cells also contain such cofactors interacting with NF1 requires further studies. In future studies, these cofactors could be identified by using the yeast two-hybrid system or by affinity chromatography using purified NF1 derivatives as immobilized ligands.

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