Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 10308–10313, August 1999
Genetics

The HNF-4/HNF-1α transactivation cascade regulates gene activity and chromatin structure of the human serine protease inhibitor gene cluster at 14q32.1

PIERRE ROLLINI AND R. E. K. FOURNIER*

Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109

Communicated by Robert N. Eisenman, Fred Hutchinson Cancer Research Center, Seattle, WA, July 7, 1999 (received for review March 17, 1999)

ABSTRACT **Hepatocyte-specific expression of the α1-antitrypsin (α1AT) gene requires the activities of two liver-enriched transactivators, hepatocyte nuclear factors 1α and 4 (HNF-1α and HNF-4). The α1AT gene maps to a region of human chromosome 14q32.1 that includes a related serine protease inhibitor (serpin) gene encoding corticosteroid-binding globulin (CBG), and the chromatin organization of this ≈130-kb region, as defined by DNase I-hypersensitive sites, has been described. Microcell transfer of human chromosome 14 from fibroblasts to rat hepatoma cells results in activation of α1AT and CBG transcription and chromatin reorganization of the entire locus. To assess the roles of HNF-1α and HNF-4 in gene activation and chromatin remodeling, we transferred human chromosome 14 from fibroblasts to rat hepatoma cell variants that are deficient in expression of HNF-1α and HNF-4. The variant cells failed to activate either α1AT or CBG transcription, and chromatin remodeling failed to occur. However, α1AT and CBG transcription could be rescued by transfecting the cells with expression plasmids encoding HNF-1α or HNF-4. In these transfectants, the chromatin structure of the entire α1AT/CBG locus was reorganized to an expressing cell-typical state. Thus, HNF-1α and HNF-4 control both chromatin structure and gene activity of two cell-specific genes within the serpin gene cluster at 14q32.1.**

The regulation of tissue-specific gene expression in mammalian cells is a complex process that involves many components. Most genes are mosaics of cis-regulatory elements, and a variety of nuclear factors, both constitutive and tissue specific, interact with these elements to modulate gene activity in a cell-specific way (1–3). Negative regulation also contributes to cell-specific patterns of gene activity (4), and the packaging of genomic DNA into chromatin is generally inhibitory to basal transcription (5). However, the molecular mechanisms that control these processes are not well understood.

The role of chromatin structure in regulating gene transcription is an area of intense interest at present. Activating a promoter in the context of chromatin requires the binding of transactivators, formation of preinitiation complexes, and initiation and elongation by RNA polymerase. All of these steps are inhibited by nucleosomes, the primary determinants of chromatin structure *in vivo* (5, 6). Multiprotein complexes exist in cells that allow specific destabilization of nucleosomes at promoters, facilitating the binding of sequence-specific factors and the general transcriptional machinery (7–10). Posttranscriptional modifications of chromatin components, particularly histone acetylation, play important roles in regulating chromatin structure and gene activity (11–15).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

PNAS is available online at www.pnas.org.

These observations indicate that studies of tissue-specific gene expression will require an analysis of regulatory mechanisms that affect chromatin structure. One aspect of chromatin structure that has been useful for identifying regulatory elements in cell-specific genes has been the mapping of DNase I-hypersensitive sites (DHSs). These are specific sites in mammalian genomes that are hypersensitive to digestion by DNase I. DHSs generally reside at cis-acting DNA elements that function in gene regulation, such as promoters, enhancers, and silencers, and they have enhanced accessibilities to trans-acting factors (16, 17). The utility of DHS mapping for identifying regulatory sites is well illustrated by the β-globin locus control region (18–22).

Hepatoma cell lines have been useful tools for studying tissue-specific gene control. Many genes are expressed specifically in the liver, and liver-specific gene expression in hepatocytes depends primarily on four families of evolutionarily conserved transcription factors—the homeodomain family of factors that are related to hepatocyte nuclear factor-1 (HNF-1), the HNF-3/forkhead (fkh) family of transactivators, the CAAT/enhancer-binding (CEBP) protein family, and the nuclear receptor/HNF-4 family (23–27). These factors function in unique, often synergistic combinations to stimulate cell-specific transcription. For example, HNF-4 (28) is a major activator of the gene encoding HNF-1α (29, 30), a member of the HNF-1 family (31–33), and HNF-1α itself activates expression of more than 20 liver genes, including α1-antitrypsin (α1AT, gene symbol *PI*) (34, 35). The expression patterns of HNF-1α and HNF-4 closely correlate with the differentiated state of cultured hepatic cells, and studies using dedifferentiated hepatoma variants, transactivator-deficient hepatoma variants, and somatic cell hybrids have suggested that the HNF-4/HNF-1α activation pathway is involved in both the determination and maintenance of hepatic phenotype (30, 35–40).

α1-Antitrypsin and corticosteroid-binding globulin (CBG) are related proteins that belong to a large family of serine protease inhibitors (serpins; for review, see ref. 41). The α1AT and CBG genes are part of a cluster of six serpin genes located on human chromosome 14q32.1, which also includes the genes encoding α1-antichymotrypsin, protein C inhibitor, kallistatin, and ATR, an antitrypsin-related sequence that may be a pseudogene (42, 43). This serpin gene cluster is organized into two discrete subclusters of three genes each, separated by 170 kb of genomic DNA (43). The serpin genes within the cluster are all highly expressed in hepatic cells, but they are not expressed in most other cell types. This provides a useful model

Abbreviations: DHS, DNase I-hypersensitive site; α1AT, α1-antitrypsin; ATR, antitrypsin-related sequence; CBG, corticosteroid-binding globulin; HNF-1α and HNF-4, hepatocyte nuclear factor 1α and 4, respectively; serpin, serine protease inhibitor; RSV, Rous sarcoma virus.

*To whom reprint requests should be addressed at: Division of Basic Sciences, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, Seattle, WA 98109. E-mail: kfournie@fhcrc.org.

system for studying gene regulation and chromatin structure within a large genomic interval.

We described recently the chromatin organization, as defined by DHSs, of an ≈130-kb region of 14q32.1 around the human α1AT and CBG genes in expressing and nonexpressing cells. Expressing cells contained 29 DHSs in this region, but only seven DHSs were found in nonexpressing cells. Furthermore, we transferred human chromosome 14 from fibroblasts to rat hepatoma cells and documented activation (44) of both α1AT and CBG transcription in the hepatoma microcell hybrids. Upon gene activation, the chromatin structure of the entire α1AT/CBG locus was reorganized so that the DHS map of the activated allele resembled that of expressing cells (45).

HNF-1α and HNF-4 are crucial for liver-specific activation of the α1AT gene (34, 46–49). Given their central roles in transcriptional activation, it was of interest to determine whether HNF-1α and/or HNF-4 were involved in regulating chromatin structure. To do this, we analyzed gene expression and chromatin structure of the α1AT/CBG locus after transfer of human chromosome 14 from fibroblasts to transactivator-deficient hepatoma variants that lacked HNF-1α and HNF-4 (35, 36, 38). In the variant cell background, the human α1AT and CBG genes were not activated, and the chromatin organization of the locus resembled that of nonexpressing cells. However, transfection of the cells with expression plasmids encoding HNF-1α or HNF-4 rescued α1AT and CBG gene expression, and the chromatin structure of the locus was reorganized to an expressing cell-typical state. These results suggest that HNF-1α and HNF-4 play important roles in controlling both gene activity and chromatin structure of the 14q32.1 serpin gene cluster.

MATERIALS AND METHODS

Cell Lines and Culture Conditions. F(14n)2 is a rat hepatoma microcell hybrid and R(14n)6 is a rat fibroblast microcell hybrid; each contains a single copy of a *neo*-marked human chromosome 14 (45). Fado-2 is a rat hepatoma line derived from H4IIEC3 (50). The generation of α1AT-*aprt*-, α1AT-*gpt*-transfected Fado-2 cells (Fg-14) and the isolation of the hepatoma variant H11 from Fg-14 cells have been described (35, 36). A *neo*-marked human chromosome 14 was introduced into H11 cells by microcell transfer (45), generating the H11(14n)D and H11(14n)E microcell hybrids. All cell lines were maintained in 1:1 Ham's F-12-DMEM (FDV) with 10% FBS (GIBCO). F(14n)2 and R(14n)6 cells were grown in medium containing 250 μg/ml G418, and the H11(14n) cell lines were cultured in medium containing 500 μg/ml G418.

Plasmid Constructs. pRSV-B1 contained coding sequences of rat HNF-1α driven by the Rous sarcoma virus (RSV) long terminal repeat (31). Plasmid pRSV-H4 was constructed by replacing the ≈3.2-kb *Hin*cII–*Bam*HI HNF-1α cDNA fragment of pRSV-B1 with the ≈2.8-kb *Bam*HI rat HNF-4 cDNA fragment from pLEN4S (28). Dual-expression plasmids containing hygromycin B and HNF-1α (pHNF-1α/Hyg) or HNF-4 (pHNF-4/Hyg) were constructed by linearizing pRSV-B1 or pRSV-H4 with *Cla*I and inserting a ≈2-kb *Cla*I–*Xho*I fragment from pPGK-*hyg* (51). These plasmids have the two transcription units, PGK-hygromycin and RSV-HNF1α or 4, in the same transcriptional orientation.

DNA Transfections. Exponentially growing H11(14n)D or H11(14n)E cells (≈10^7cells per transfection) were harvested and suspended in 1 ml of ice-cold FDV, and 10 μg of *Sfi*I-linearized pHNF-1α/Hyg or pHNF-4/Hyg was added. The cells were electroporated at 960 μF and 300 V by using a Bio-Rad Gene Pulser. Cells were incubated for ≈30 h in nonselective medium, and selective medium containing 300 μg/ml hygromycin B was added. Clones were isolated after 3 weeks. Subclones were prepared by inoculating ≈100–500 cells into 100-mm plates and picking subclones after 3 weeks.

RNA Analysis. Cytoplasmic RNAs were isolated and analyzed on agarose-formaldehyde gels as described (45). The probes used for RNA blot hybridization were: α1AT, a 567-bp *Bam*HI–*Dra*I fragment from cosmid αATc1 (52) that contained most exon II sequences of α1AT; CBG, a 1.46-kb *Eco*RI fragment containing full-length human CBG cDNA (53); HNF-1α, a ≈3.2-kb *Hin*cII–*Bam*HI fragment from pRSV-B1 (31) containing rat HNF-1α cDNA; HNF-4, a ≈2.8-kb *Bam*HI fragment from pLEN4S (28) containing rat HNF-4 cDNA; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a ≈1.2-kb *Pst*I fragment containing human GAPDH cDNA (54).

DNase I Hypersensitive Site Mapping and Generalized DNase I-Sensitivity Assays. Nuclei were isolated, digested with DNase I, and analyzed by Southern hybridization by using a variety of unique-sequence probes from the α1AT-ATR-CBG region (42, 43) as described (45). A similar range of DNase I concentrations was used for each cell line.

RESULTS

Rat Hepatoma Variant H11 Does Not Activate Human α1AT or CBG Transcription After Chromosome Transfer. The human serpin gene cluster at 14q32.1 is composed of two subclusters of three genes, each separated by ≈170 kb of genomic DNA (43). The proximal subcluster contains α1AT, ATR, and CBG, and the genes are arranged in a head-to-tail orientation in the order *tel*-α1AT-ATR-CBG-*cen*. There is ≈12 kb of genomic DNA between α1AT and ATR and ≈40 kb between ATR and CBG. When we transferred human chromosome 14 from fibroblasts to rat hepatoma cells, α1AT and CBG transcription was activated, and the chromatin structure of the entire ≈130-kb region was reorganized from a nonexpressing to an expressing cell-typical state (45). To determine the roles of cell-specific transactivators in these processes, we transferred human chromosome 14 to H11, a rat hepatoma variant that is deficient in expression of HNF-1α and HNF-4 (36).

The H11 variant cell line was derived from Fado-2 ($APRT^-$, $HPRT^-$) rat hepatoma cells in three steps. Fado-2 cells were transfected sequentially with expression plasmids in which murine adenine phosphoribosyltransferase (*aprt*) and bacterial xanthine-guanine phosphoribosyltransferase (*gpt*) coding sequences were driven by a 640-bp promoter fragment from the human α1AT gene. The resulting transfectants expressed APRT and GPT activities from stably integrated transgenes that were inserted at different chromosomal sites. To isolate variant sublines, $APRT^-$, GPT^- cells were selected in medium containing 2,6-diaminopurine (DAP) and 6-thioxanthine (6TX). Most of the doubly selected variants that were obtained displayed deficiency phenotypes in trans, because their chromosomal α1AT genes were inactive. These variant lines were deficient in the expression of HNF-1α and HNF-4 (35, 36). Other HNF-1α- or HNF-4-dependent liver genes also were inactive in the variants, but liver genes whose expression was independent of HNF-1α and HNF-4 continued to be expressed. H11 variant cells and their derivatives displayed characteristic hepatic morphologies *in vitro*, suggesting that H11 cells are hepatic cells that are specifically deficient in the HNF-4/HNF-1α transactivation pathway (35, 36).

We transferred a *neo*-marked human chromosome 14 from HDm-5, a mouse fibroblast/human microcell hybrid, to H11 cells by microcell fusion. H11(14n)D and H11(14n)E were independent microcell hybrid clones that each contained an intact copy of human chromosome 14 as assessed by fluorescence *in situ* hybridization and Southern analysis using probes from the 14q32.1 serpin gene cluster (42, 43) and the cathepsin G gene (55) at 14q11.2 (not shown). The morphologies of these microcell hybrids were typically hepatic.

Microcell transfer of human chromosome 14 from fibroblasts to wild-type rat hepatoma cells generates microcell hybrids in which human α1AT and CBG transcription is activated (45). For example, F(14n)2 cells are rat hepatoma cells that contain a single copy of human chromosome 14, and human α1AT and CBG mRNAs are expressed at hepatoma-typical levels in these cells (Fig. 1). In contrast, transfer of human chromosome 14 from fibroblasts to H11 variant cells produced microcell hybrids [H11(14n)D, H11(14n)E] that failed to activate either α1AT or CBG mRNA transcription, because no human α1AT or CBG transcripts were apparent even after prolonged exposure of the RNA blots (Fig. 1). Like their H11 parent, neither of these microcell hybrids expressed HNF-1α or HNF-4 mRNAs (Fig. 1). Thus, the transferred human α1AT and CBG genes were regulated in trans in hepatoma microcell hybrids, and activation of both genes required HNF-1α and HNF-4.

Chromatin Organization of the Human α1AT/ATR/CBG Locus in H11 Variant Cells. We previously mapped DHSs in a ≈130-kb segment of the human α1AT-ATR-CBG region in expressing and nonexpressing cells (45). Human hepatoma ($HepG_2$) cells contained 29 DHSs in this interval, but only seven of those sites were found in nonexpressing (HeLa) cells. Furthermore, transfer of human chromosome 14 from fibroblasts to wild-type rat hepatoma cells resulted in activation of human α1AT and CBG transcription and reorganization of the entire locus to an expressing cell-typical chromatin state. To assess the roles of HNF-1α and HNF-4 in chromatin remodeling, we mapped DHSs in H11-derived microcell hybrids that contained human chromosome 14.

Nuclei from H11(14n)D and H11(14n)E cells were incubated with DNase I, and DNA was purified and analyzed by Southern hybridization by using a variety of probes from the α1AT-ATR-CBG region, as described (45). One experiment is shown in Fig. 2*A*, where two expression-associated DHSs ≈19 and ≈21 kb upstream of the α1AT gene [position zero is the *Eco*RI site in macrophage-specific exon I_A (42)] were observed in the activated α1AT-ATR-CBG allele of F(14n)2 cells. In contrast, neither of these sites was apparent when human chromosome 14 was transferred to rat fibroblasts [R(14n)6] or to transactivator-deficient hepatoma variants [H11(14n)E, Fig. 2A]. Another example is shown in Fig. 2*B*, with two sites at ≈+37 kb found in F(14n)2 cells, but only one weak site in H11(14n)D, H11(14n)E, and R(14n)6. Similar DHS mapping experiments were performed throughout the ≈130-kb α1AT-ATR-CBG region.

The distributions of DHSs in the human α1AT-ATR-CBG locus in different hybrid cell types are summarized in Fig. 3. As shown previously, human α1AT and CBG gene expression was activated in F(14n)2 cells, a rat hepatoma hybrid that contains human chromosome 14, and the chromatin structure of the entire α1AT-ATR-CBG locus was reorganized such that 16 of 22 expression-associated DHSs of human hepatoma ($HepG_2$) cells were induced (45). In contrast, R(14n)6 cells, a rat fibroblast microcell hybrid that contains human chromosome 14, failed to express α1AT and CBG, and the α1AT-ATR-CBG region contained only six constitutive DHSs.

The DHS maps of the α1AT-ATR-CBG locus in the H11-derived microcell hybrids [H11(14n)D, H11(14n)E] were similar to those of nonexpressing cells. For example, the extinguished α1AT-ATR-CBG allele of R(14n)6 cells contained only six constitutive DHSs; H11(14n)D and E displayed five of those sites plus another site ≈22 kb upstream of α1AT. Among the constitutive sites, one was near the α1AT macrophage promoter (56), one was in intron 3 of α1AT, two were between ATR and CBG, and one was in CBG intron 3. These five constitutive DHSs also were present in nonexpressing human

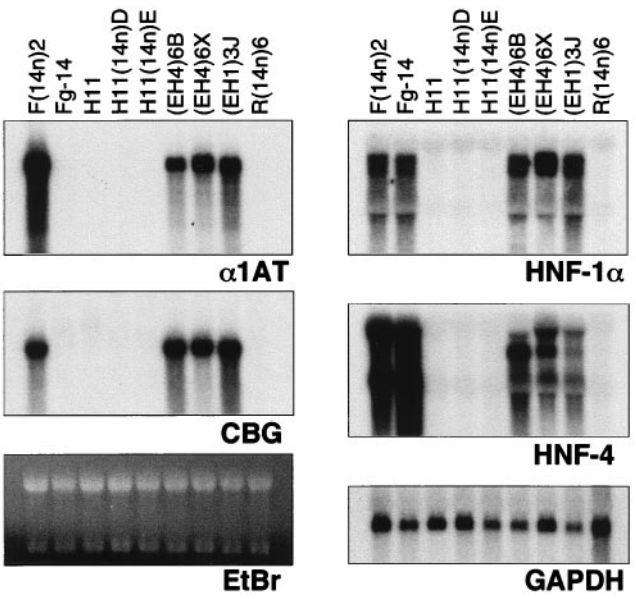

FIG. 1. Expression of α1AT, CBG, HNF-1α, and HNF-4 mRNAs in parental cells, microcell hybrids, and transfectants. Cytoplasmic RNAs (8 μg/lane) from a rat hepatoma hybrid containing human chromosome 14 [F(14n)2], Fg-14 rat hepatoma cells, the H11 rat hepatoma variant, H11 variants containing human chromosome 14 [H11(14n)D and H11(14n)E], rescued variants expressing HNF-4 [(EH4)6B, (EH4)6X] or HNF-1α [(EH1)3J], and a rat fibroblast microcell hybrid containing human chromosome 14 [R(14n)6] were analyzed by Northern blot hybridization. The α1AT and CBG probes used were human cDNAs that did not cross-hybridize to rat mRNAs under the conditions used. Loading controls were ethidium bromide (EtBr)-stained gels and hybridization with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe (54).

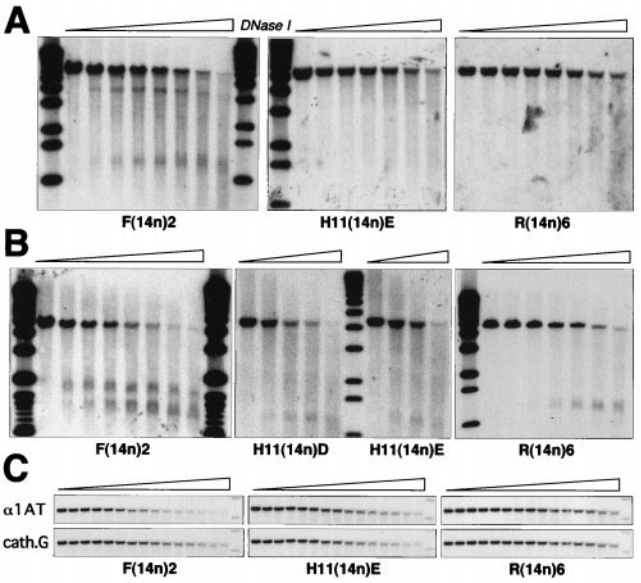

FIG. 2. DHS mapping and generalized DNase I-sensitivity assays in microcell hybrids. Nuclei from human chromosome 14-containing rat hepatoma [F(14n)2], variant [H11(14n)D, H11(14n)E], and fibroblast [R(14n)6] cells were digested with DNase I, and DNA was purified, digested with *Bam*HI (*A*), *Bgl*II (*B*), or *Xba*I (*C*) and analyzed by Southern hybridization. The probes for the DHS mapping experiments were a 0.7-kb *Eco*RI–*Hin*dIII fragment from map position ≈-21.5 kb (*A*) and a ≈0.95-kb *Bgl*II–*Ava*I fragment from map position ≈+36 kb (*B*). The probes for the generalized DNase I-sensitivity assays (*C*) were a 1.4-kb *Xho*I–*Hin*dIII genomic fragment containing exon III of the α1AT gene from cosmid αATc1 (52) and a ≈1.0-kb *Xba*I–*Pst*I genomic fragment containing exon II from cathepsin G (55). Both probes hybridized with DNA fragments of ≈2.4 kb in *Xba*I digests of genomic DNA.

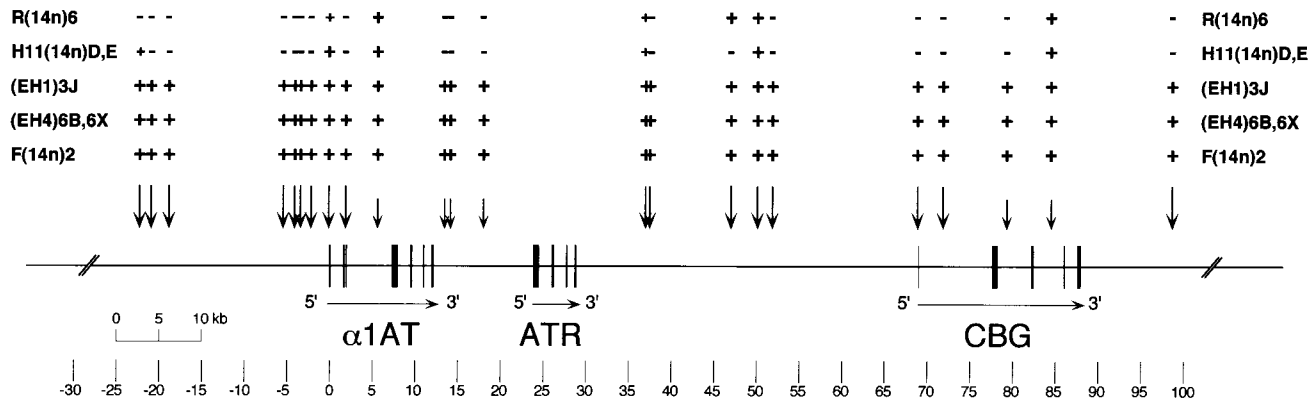

FIG. 3. DHS map of activated, extinguished, variant, and rescued α1AT-ATR-CBG alleles. The map is drawn to scale, with position zero defined as the *Eco*RI site in macrophage-specific exon I_A of α1AT (42). Exons are indicated as black boxes, with arrows below the map showing the transcriptional orientations of the genes. All DHSs of the ≈130-kb region are depicted above the map as vertical arrows. Long arrows indicate strong DHSs, and short arrows indicate weak sites in F(14n)2 cells. The presence or absence of specific DHSs in an activated allele [F(14n)2], an extinguished allele [R(14n)6], variant alleles [H11(14n)D and E], and rescued alleles [HNF-1α, (EH1)3J; HNF-4, (EH4)6B, 6X] is indicated above the map by + or − signs. A small + indicates a DHS weaker than that of the activated allele of F(14n)2.

(HeLa) cells (45). The variant allele did not display a constitutive DHS at +47 kb, between ATR and CBG, but it had a weak constitutive site at ≈−22 kb that was present in HeLa cells (45). Most notably, however, none of the 16 expression-associated DHSs that were induced upon gene activation in wild-type rat hepatoma cells were formed in the H11 variant cell background.

To determine whether HNF-1α and/or HNF-4 affected the overall organization of α1AT chromatin, generalized DNase I sensitivity tests were performed by using probes from regions that were devoid of DHSs (Fig. 2*C*). In F(14n)2 cells, α1AT gene sequences were relatively sensitive to DNase I digestion compared with sequences from the nonexpressed cathepsin G gene at 14q11.2. In contrast, α1AT and cathepsin G gene sequences were similarly DNase I-resistant in R(14n)6 and H11(14n)E cells. Thus, both DHS mapping and generalized nuclease sensitivity tests indicate that HNF-1α and HNF-4 were required for both gene activation and chromatin remodeling of the α1AT-ATR-CBG region.

Rescue of Human α1AT and CBG Gene Expression by HNF-1α or HNF-4. H11 variant cells are hepatic cells that express many liver-specific genes, but they are deficient in HNF-4/HNF-1α-mediated transactivation (36). Furthermore, HNF-4/HNF-1α-dependent liver gene expression can be rescued by transfecting H11 cells with expression plasmids encoding HNF-4 or HNF-1α (35, 36, 38). Therefore, we used H11 microcell hybrids containing human chromosome 14 to determine the extent of α1AT and CBG gene activation and chromatin remodeling after transfection with HNF-1α or HNF-4.

Dual-expression plasmids encoding the hygromycin B resistance gene and either HNF-1α or HNF-4 were introduced into H11(14n)D and H11(14n)E cells by electroporation. Hygromycin B-resistant clones were isolated, and expression of the introduced HNF-1α and HNF-4 expression cassettes was assessed by RNA blot hybridization. Only a subset of the hygromycin B-resistant clones expressed HNF-1α or HNF-4. For example, some HNF-1α transfectants expressed wild-type levels of HNF-1α, but others failed to accumulate detectable HNF-1α mRNA. Similarly, only a fraction of the HNF-4 transfectants expressed HNF-4 mRNA. In general, <50% of the clones expressed transactivator mRNAs, and transactivator mRNA expression in many of the transfectant clones was unstable (data not shown).

Transfectants (EH1)3 (an HNF-1α transfectant) and (EH4)6 (an HNF-4 transfectant) were subcloned to generate (EH1)3J, (EH4)6B, and (EH4)6X. These subclones stably expressed the transfected transactivator mRNAs (Fig. 1). The HNF-4 transfectants [(EH4)6B and 6X] reactivated their chromosomal HNF-1 and CBG genes to near wild-type levels in response to HNF-4; α1AT expression also was activated, but not to wild-type levels (Fig. 1). The HNF-1α transfectant [(EH1)3J] rescued CBG and, to a lesser extent, α1AT and HNF-4 mRNA expression. These results were in accord with previous studies that suggested that HNF-4 could activate the HNF-1α promoter and vice versa, and both factors were required for high-level α1AT gene expression (29, 30, 35). Thus, HNF-1α and/or HNF-4 were sufficient to reactivate expression of both human α1AT and CBG in the H11 microcell hybrid variant background. We next determined whether chromatin reorganization accompanied gene activation.

Chromatin Reorganization of the Human α1AT-ATR-CBG Locus in Transactivator-Rescued Microcell Hybrids. DHS mapping experiments were performed in transactivator-rescued microcell hybrids [(EH1)3J, (EH4)6B, (EH4)6X] to determine whether chromatin reorganization accompanies HNF-1α- and HNF-4-mediated gene activation. Three DHS mapping experiments are shown in Fig. 4, where the chromatin structures of inactive variant alleles (Fig. 4 *Left*) and rescued alleles (Fig. 4 *Right*) are compared. For example, one constitutive DHS in the macrophage-specific α1AT promoter (Fig. 4*A*, open arrow) was present in all of the hybrid cell lines, but the rescued variants also contained an expression-associated site (position ≈+1.9 kb) in the hepatocyte-specific α1AT promoter (Fig. 4*A*, solid arrow). Fig. 4*B* shows expression-associated DHSs in the promoter and first intron of CBG (positions ≈+69 and +72 kb); the variant allele did not contain these DHSs, but they were induced in the rescued variants. In Fig. 4*C*, the region 3–6 kb upstream of α1AT was analyzed; it contained three DHSs in the rescued variants (positions ≈−3.4 kb, −4.1 kb, and −5.5 kb). None of these sites was present in the variant allele. The complete DHS maps of the three rescued variants are shown in Fig. 3.

As summarized in Fig. 3, rescue of human α1AT and CBG gene expression by transfecting the variant hybrids with HNF-1α or HNF-4 resulted in reorganization of α1AT-ATR-CBG chromatin over a region of >100 kb. In the H11(14n)D and H11(14n)E variant hybrids, the locus displayed a chromatin configuration like that of nonexpressing cells. After transactivator rescue, the chromatin structure of the entire locus was reorganized such that it was indistinguishable from that of the active locus in F(14n)2 cells; that is, all 16 expression-associated DHSs were induced by expression of either HNF-1α or HNF-4. Thus, long-range chromatin reorganization of the

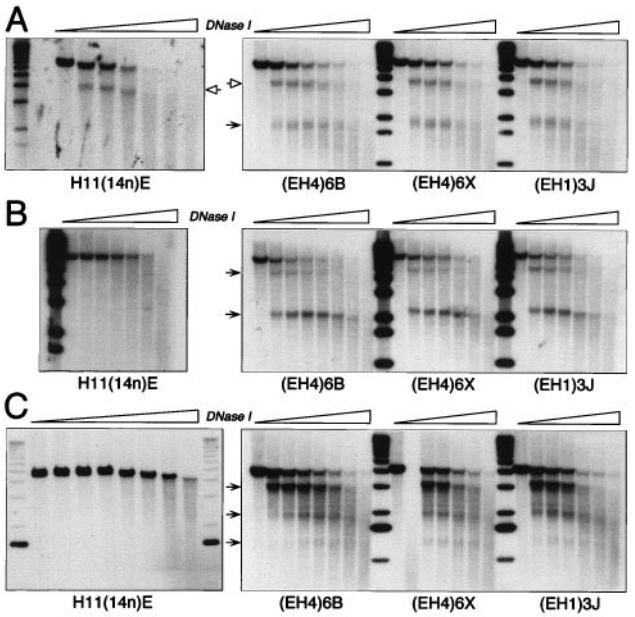

FIG. 4. HNF-1α or HNF-4 induces long-range chromatin reorganization of the human α1AT-ATR-CBG locus in H11 variant cells. Nuclei from an H11 variant containing human chromosome 14 [H11(14n)E] and rescued variant transfectants expressing HNF-4 [(EH4)6B, (EH4)6X] or HNF-1α [(EH1)3J] were treated with DNase I, and isolated DNA was cleaved with *Hin*dIII (*A* and *B*) or *Eco*RI (*C*). The probes were a 0.9-kb *Spe*I–*Hin*dIII fragment (+2.7 to +3.6 kb) (*A*); a 0.6-kb *Bgl*II fragment (+67.7 to +68.3 kb) (*B*); and a 2.2-kb *Hin*dIII fragment (−4.9 to −2.7 kb) (*C*). Solid arrows indicate DHSs that were induced in the rescued variants. Open arrows in *A* indicate a constitutive DHS that was found in all cell lines.

α1AT-ATR-CBG locus accompanies α1AT and CBG activation. These data indicate that HNF-1α and HNF-4 play important roles not only in activating human α1AT and CBG transcription, but also in establishing the proper expression-associated, long-range chromatin structure of the α1AT/ATR/CBG region.

DISCUSSION

Somatic cell variants with defects in expression of tissue-specific genes are useful genetic tools. Many of the rat hepatoma variants that were isolated previously were recognized based on altered cell morphologies; these "dedifferentiated" lines displayed pleiotrophic phenotypes that were dominant in cell hybrids (57, 58). We isolated rat hepatoma variants with specific, trans-acting lesions by selecting cells that were unable to transactivate two unlinked α1AT promoters (36). Because liver-specific expression of α1AT depends on HNF-1α and HNF-4 (34, 46–49), variants deficient in expression of the two transactivators were obtained. These variant lines continued to express liver-specific genes whose expression was independent of HNF-1α and HNF-4, and they displayed characteristic hepatic morphologies. Furthermore, the variant phenotypes could be rescued by transfecting the cells with cloned transactivator genes, and they were recessive in cell hybrids (35, 36, 38). These variant lines provide a system to assess the roles of HNF-1α and HNF-4 in regulating gene expression and chromatin structure.

Chromatin structure plays an important role in regulating gene expression (5). One aspect of chromatin structure that has been useful for identifying cis-regulatory regions has been the mapping of DHSs (16, 17). We recently mapped DHSs in a ≈130-kb region of chromosome 14q32.1 that includes the human α1AT and CBG genes (45). These genes are actively transcribed in hepatic cells but repressed in most cell types. The distribution of DHSs in expressing and nonexpressing cells was very different, with 29 DHSs in expressing cells but only seven sites in nonexpressing cells. Furthermore, microcell transfer of human chromosome 14 from fibroblasts to rat hepatoma cells resulted in activation of human α1AT and CBG transcription and reorganization of the chromatin structure of the entire locus from a nonexpressing to an expressing cell-typical state (45). These data indicate that the terminally differentiated hepatoma and fibroblast recipient cells used in these chromosome transfer experiments contained all the regulatory factors required for both the establishment and maintenance of cell-specific patterns of gene activity and long-range chromatin structure, and that germ-line and/or lineage-specific events were not required.

To assess the roles of HNF-1α and HNF-4 in gene activation and chromatin remodeling, we transferred human chromosome 14 to a transactivator-deficient hepatoma cell variant. Neither human α1AT nor CBG gene expression was activated in H11 variant cells. Thus, in addition to the well-established roles of these transactivators in activating the α1AT promoter (34, 46–49), HNF-1α and/or HNF-4 are required for CBG gene expression as well. This is consistent with the identification of potential binding sites for HNF-1 in the CBG promoter (59, 60).

The chromatin organization of the human α1AT-ATR-CBG locus in H11 variant cells resembled that of nonexpressing cells. Specifically, the DHS map of the entire ≈130-kb region was similar to that of an extinguished allele in a rat fibroblast microcell hybrid: only six constitutive DHSs were formed. Moreover, the generalized DNase I sensitivity of α1AT gene sequences in the H11 background resembled that of nonexpressing cells. Thus, gene activation and chromatin remodeling of the α1AT-ATR-CBG locus both require HNF-1α, HNF-4, or other unidentified factors that are deficient in H11 variant cells.

To assess more directly the roles of HNF-1α and HNF-4 in chromatin remodeling, variant microcell hybrids were transfected with expression plasmids encoding HNF-1α or HNF-4. Stable transfectants expressing HNF-1α and HNF-4 were obtained by subcloning, and gene activity and chromatin structure of the α1AT-ATR-CBG locus were assessed. Expression of both α1AT and CBG was rescued in the transfectants, and this was accompanied by the formation of expression-associated DHSs throughout the ≈130-kb region. We presume that generalized DNase I sensitivity in the interval also was rescued to an expression-typical state, but these assays will require the isolation of homogeneous populations of transfectant cells expressing wild-type levels of α1AT and CBG mRNAs. Nonetheless, it is clear that HNF-1α and HNF-4 not only control cell-specific transactivation of α1AT, CBG, and other liver genes, but they also play important roles in regulating chromatin structure. Whether the functions of HNF-1α and HNF-4 in chromatin remodeling are distinct from their roles in activating transcription is not known, and those functions could be direct or indirect. Furthermore, the relative roles of the two transactivators in controlling these processes are difficult to assess because of autoregulation between HNF-1α and HNF-4 (29, 30, 35, 61).

Ectopic expression of HNF-1α and/or HNF-4 does not activate transcription of downstream genes in nonexpressing cells. For example, the chromosomal α1AT genes of rat fibroblasts and hepatoma × fibroblast hybrids are refractory to activation by HNF-1α and HNF-4 (34, 35, 62). Similarly, HNF-4 fails to activate HNF-1α expression in fibroblasts or HeLa cells. These phenotypes are very different from those of transactivator-deficient hepatoma variants, in which both gene activity and chromatin organization of the α1AT-ATR-CBG region are responsive to HNF-1α and HNF-4. We presume that nonexpressing cells contain factors that prevent chromatin remodeling of the α1AT-ATR-CBG locus or that hepatoma

cells contain factors required for chromatin remodeling. Genetic complementation tests using mutant alleles of α1AT or CBG that encode selectable markers (63) may be a useful approach for identifying these regulatory factors.

How could HNF-1α and HNF-4 be involved in chromatin remodeling? One current view is that cell-specific transactivators function in chromatin remodeling by recruiting chromatin-modifying complexes to transcriptionally active genes. For example, some transcriptional activators can direct histone acetyltransferase complexes to nucleosomes (64). In this model, transcription *per se* affects chromatin structure. If HNF-1α and/or HNF-4 function in this way, increasing histone acetylation around the α1AT and CBG genes, one might expect that inhibitors of histone deacetylases might alter α1AT-ATR-CBG chromatin structure in H11 cells much like HNF-1α/HNF-4 transactivator rescue. It is also possible that HNF-1α and/or HNF-4 are components of chromatin-modifying complexes, and their functions in these complexes might be distinct from their roles as transcriptional activators. Finally, α1AT-ATR-CBG chromatin reorganization in variant cells transfected with HNF-1α or HNF-4 might be indirect effects mediated by factors that are induced by HNF-1α or HNF-4, not by the transactivators themselves. These possibilities will be interesting to explore.

The DHS map of the human α1AT-ATR-CBG locus in transactivator-rescued variants was indistinguishable from that of expressing cells, and 16 expression-associated DHSs were induced after transfection with either HNF-1α or HNF-4. A few of these sites, like those in the α1AT and CBG promoters (positions $\approx$+1.9 and +68.9 kb), probably were formed by the direct binding of HNF-1α and/or HNF-4. It is unlikely that all of the other expression-associated DHSs are binding sites for HNF-1α and/or HNF-4, so the roles of the factors in the formation of these DHSs are likely to be indirect, and other unidentified factors probably are involved. The construction and analysis of specific mutant alleles of the α1AT-ATR-CBG locus by homologous recombination (65) should allow the functions of individual elements in controlling gene activity and chromatin structure to be defined, and somatic cell variants will be one useful cell background for functional tests.

We thank Mark Marsden for help with the microcell fusions, Gary Bulla for pRSV-H4, and Mark Groudine and Steve Tapscott for their comments on the manuscript. These studies were supported by Grant GM26449 from the National Institute of General Medical Sciences. P.R. was supported by fellowships from the Swiss National Science Foundation, the Swiss League against Cancer, and the Schweizerische Stiftung für Medizinisch-Biologische Stipendien.

1. Johnson, P. F. & McKnight, S. L. (1989) *Annu. Rev. Biochem.* **58,** 799–839.
2. Mitchell, P. J. & Tjian, R. (1989) *Science* **245**, 371–378.
3. Tjian, R. & Maniatis, T. (1994) *Cell* **77,** 5–8.
4. Hanna-Rose, W. & Hansen, U. (1996) *Trends Genet.* **12,** 229–234.
5. Felsenfeld, G. (1992) *Nature (London)* **355,** 219–224.
6. Adams, C. C. & Workman, J. L. (1993) *Cell* **72,** 305–308.
7. Kingston, R. E., Bunker, C. A. & Imbalzano, A. N. (1996) *Genes Dev.* **10,** 905–920.
8. Peterson, C. L. (1996) *Curr. Opin. Genet. Dev.* **6,** 171–175.
9. Svaren, J. & Hörz, W. (1996) *Curr. Opin. Genet. Dev.* **6,** 164–170.
10. Tsukiyama, T. & Wu, C. (1997) *Curr. Opin. Genet. Dev.* **7,** 182–191.
11. Brownell, J. E. & Allis, C. D. (1996) *Curr. Opin. Genet. Dev.* **6,** 176–184.
12. Grunstein, M. (1997) *Nature (London)* **389,** 349–352.
13. Wolffe, A. P., Wong, J. & Pruss, D. (1997) *Genes Cells* **2,** 291–302.
14. Kadonaga, J. T. (1998) *Cell* **92,** 307–313.
15. Struhl, K. (1998) *Genes Dev.* **12,** 599–606.
16. Elgin, S. C. R. (1988) *J. Biol. Chem.* **263,** 19259–19262.
17. Gross, D. S. & Garrard, W. T. (1988) *Annu. Rev. Biochem.* **57,** 159–197.
18. Tuan, D., Solomon, W., Li, Q. & London, I. M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6384–6388.
19. Forrester, W. C., Thompson, C., Elder, J. T. & Groudine, M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1359–1363.
20. Forrester, W. C., Takegawa, S., Papayannopoulou, T., Stamatoyannopoulos, G. & Groudine, M. (1987) *Nucleic Acids Res.* **15,** 10159–10177.
21. Grosveld, F., van Assendelft, G. B., Greaves, D. R. & Kollias, G. (1987) *Cell* **51,** 975–985.
22. Kioussis, D. & Festenstein, R. (1997) *Curr. Opin. Genet. Dev.* **7,** 614–619.
23. De Simone, V. & Cortese, R. (1991) *Curr. Opin. Cell Biol.* **3,** 960–965.
24. Lai, E. & Darnell, J. E., Jr. (1991) *Trends Biochem. Sci.* **16,** 427–430.
25. Sladek, F. M. & Darnell, J. E., Jr. (1992) *Curr. Opin. Genet. Dev.* **2,** 256–259.
26. Xanthopoulos, K. G. & Mirkovitch, J. (1993) *Eur. J. Biochem.* **216,** 353–360.
27. Cereghini, S. (1996) *FASEB J.* **10,** 267–282.
28. Sladek, F. M., Zhong, W., Lai, E. & Darnell, J. E., Jr. (1990) *Genes Dev.* **4,** 2353–2365.
29. Tian, J.-M. & Schibler, U. (1991) *Genes Dev.* **5,** 2225–2234.
30. Kuo, C. J., Conley, P. B., Chen, L., Sladek, F. M., Darnell, J. E., Jr., & Crabtree, G. R. (1992) *Nature (London)* **355,** 457–461.
31. Frain, M., Swart, G., Monaci, P., Nicosia, A., Stämpfli, S., Frank, R. & Cortese, R. (1989) *Cell* **59,** 145–157.
32. Mendel, D. B. & Crabtree, G. R. (1991) *J. Biol. Chem.* **266,** 677–680.
33. Tronche, F. & Yaniv, M. (1992) *BioEssays* **14,** 579–587.
34. Bulla, G., DeSimone, V., Cortese, R. & Fournier, R. E. K. (1992) *Genes Dev.* **6,** 316–327.
35. Bulla, G. A. & Fournier, R. E. K. (1994) *Mol. Cell. Biol.* **14,** 7086–7094.
36. Bulla, G. A. & Fournier, R. E. K. (1992) *Somat. Cell Mol. Genet.* **18,** 361–370.
37. Griffo, G., Hamon-Benais, C., Angrand, P.-O., Fox, M., West, L., Lecoq, O., Povey, S., Cassio, D. & Weiss, M. C. (1993) *J. Cell Biol.* **121,** 887–898.
38. Bulla, G. A. (1997) *Somat. Cell Mol. Genet.* **23,** 185–201.
39. Chaya, D., Fougère-Deschatrette, C. & Weiss, M. C. (1997) *Mol. Cell. Biol.* **17,** 6311–6320.
40. Späth, G. F. & Weiss, M. C. (1997) *Mol. Cell. Biol.* **17,** 1913–1922.
41. Potempa, J., Korzus, E. & Travis, J. (1994) *J. Biol. Chem.* **269,** 15957–15960.
42. Rollini, P. & Fournier, R. E. K. (1997) *Mamm. Genome* **8,** 913–916.
43. Rollini, P. & Fournier, R. E. K. (1997) *Genomics* **46,** 409–415.
44. Gourdeau, H. & Fournier, R. E. K. (1990) *Annu. Rev. Cell Biol.* **6,** 69–94.
45. Rollini, P. & Fournier, R. E. K. (1999) *Genomics* **56,** 22–30.
46. Courtois, G., Morgan, J. G., Campbell, L. A., Fourel, G. & Crabtree, G. R. (1987) *Science* **238,** 688–692.
47. Li, Y., Shen, R.-F., Tsai, S. Y. & Woo, S. L. C. (1988) *Mol. Cell Biol.* **8,** 4362–4369.
48. Monaci, P., Nicosia, A. & Cortese, R. (1988) *EMBO J.* **7,** 2075–2087.
49. Costa, R. H., Grayson, D. R. & Darnell, J. E., Jr. (1989) *Mol. Cell. Biol.* **9,** 1415–1425.
50. Pitot, H. C., Peraino, C., Morse, P. A. & Potter, V. R. (1964) *Natl. Cancer Inst. Monogr.* **13,** 229–242.
51. te Riele, H., Maandag, E. R., Clarke, A., Hooper, M. & Berns, A. (1990) *Nature (London)* **348,** 649–651.
52. Kelsey, G. D., Povey, S., Bygrave, A. E. & Lovell-Badge, R. H. (1987) *Genes Dev.* **1,** 161–171.
53. Hammond, G. L., Smith, C. L., Goping, I. S., Underhill, D. A., Harley, M. J., Reventos, J., Musto, N. A., Gunsalus, G. L. & Bardin, C. W. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5153–5157.
54. Tso, J. Y., Sun, X.-H., Kao, T., Reece, K. S. & Wu, R. (1985) *Nucleic Acids Res.* **13,** 2485–2502.
55. Hohn, P. A., Popescu, N. C., Hanson, R. D., Salvesen, G. & Ley, T. J. (1989) *J. Biol. Chem.* **264,** 13412–13419.
56. Hafeez, W., Ciliberto, G. & Perlmutter, D. H. (1992) *J. Clin. Invest.* **89,** 1214–1222.
57. Deschatrette, J. & Weiss, M. C. (1974) *Biochimie* **56,** 1603–1611.
58. Deschatrette, J., Moore, E. E., Dubois, M., Cassio, D. & Weiss, M. C. (1979) *Somat. Cell Genet.* **5,** 697–718.
59. Underhill, D. A. & Hammond, G. L. (1989) *Mol. Endocrinol.* **3,** 1448–1454.
60. Underhill, D. A. & Hammond, G. L. (1995) *Gene* **162,** 205–211.
61. Zhong, W., Mirkovitch, J. & Darnell, J. E., Jr. (1994) *Mol. Cell. Biol.* **14,** 7276–7284.
62. Bulla, G. A. (1997) *Nucleic Acids Res.* **25,** 2501–2508.
63. Porter, M. B. & Fournier, R. E. K. (1996) *Somat. Cell Mol. Genet.* **22,** 311–327.
64. Utley, R. T., Ikeda, K., Grant, P. A., Côté, J., Steger, D. J., Eberharter, A., John, S. & Workman, J. L. (1998) *Nature (London)* **394,** 498–502.
65. Dieken, E. S., Epner, E. M., Fiering, S., Fournier, R. E. K. & Groudine, M. (1996) *Nat. Genet.* **12,** 174–182.