Characterization of a Cofactor That Regulates Dimerization of a Mammalian Homeodomain Protein

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Dimerization among transcription factors has become a recurrent theme in the regulation of eukaryotic gene expression. Hepatocyte nuclear factor–1α (HNF-1α) is a homeodomain-containing protein that functions as a dimer. A dimerization cofactor of HNF-1α (DCoH) was identified that displayed a restricted tissue distribution and did not bind to DNA, but, rather, selectively stabilized HNF-1α dimers. The formation of a stable tetrameric DCoH–HNF-1α complex, which required the dimerization domain of HNF-1α, did not change the DNA binding characteristics of HNF-1α, but enhanced its transcriptional activity. However, DCoH did not confer transcriptional activation to the GAL4 DNA binding domain. These results indicate that DCoH regulates formation of transcriptionally active tetrameric complexes and may contribute to the developmental specificity of the complex.

IN COMPLEX BIOLOGICAL SYSTEMS, A LARGE NUMBER OF processes appear to be precisely controlled with a limited number of regulatory molecules. Several general schemes have emerged that suggest ways to overcome this limitation, including variation in the activity of regulatory proteins and the combinatorial use of regulatory factors to extend the range and diversity of control (*1*).

Homo- and heterodimerization occur among members of a family of transcription factors that share a common dimerization domain and have been recognized as a means of diversification of the degree of regulatory control exerted through a single DNA binding site. The dimerization of transcription factors, which has been most extensively studied among proteins that contain the leucine zipper (coiled-coil) and helix-loop-helix DNA binding and dimerization motifs (*2*), generally brings about a change in transcriptional activity, often mediated by a change in sequence specificity or degree of activation. Despite the obvious advantages of regulating the dimerization process, cellular accessory proteins that perform this function have not been identified.

A dimerization motif has been found in the transcription factor hepatocyte nuclear factor–1α (HNF-1α, also referred to as HNF-1, LFB1, and APF) (*3*). This homeodomain protein regulates the expression of a large number of genes expressed primarily in the liver, but also in a complex array of endodermally and mesodermally derived tissues including the intestine and kidney (*4*). Unlike other homeodomain-containing proteins, HNF-1α must dimerize in order to bind to DNA. The dimerization domain is composed of the 32 amino-terminal amino acid residues of HNF-1α (*5*). Although HNF-1α dimerizes on its own, there is evidence that a tissue-restricted factor might regulate the dimerization. First, native HNF-1α dimers in liver nuclear extracts are stable (*6*), whereas the subunits of dimers produced by in vitro translation or expressed in mammalian cells that do not normally contain HNF-1α are freely exchangeable within minutes at room temperature (*5, 6*). Second, the ability of HNF-1α to induce transcription of HNF-1–dependent reporter constructs in transient transfection assays varies, depending on the recipient cell used (*7*).

We now report the purification, cloning, and characterization of a protein, dimerization cofactor for HNF-1 (DCoH), that copurifies with HNF-1α from rat liver nuclear extracts as a result of its ability to bind to HNF-1α.

Purification and cloning of DCoH. A two-step procedure based on DNA affinity chromatography was originally used to purify HNF-1α to apparent homogeneity from rat liver nuclear extract (*8*). Although the purified HNF-1α migrated as a single broad band on SDS polyacrylamide gels, it consistently eluted from a C8 reversed-phase HPLC (high-performance liquid chromatography) column as a single major peak, with a shoulder on the leading edge. The observation that the size of the shoulder relative to that of the major peak does not vary between experiments suggested that the shoulder might represent a second protein component. By denaturing the purified HNF-1α and subjecting it to pyridinylation prior to loading onto the C8 column, we resolved the shoulder from the major peak. Microsequencing and SDS–polyacrylamide gel electrophoresis (SDS-PAGE) of the protein components of the resolved peaks indicated that the major peak contained exclusively HNF-1α and that the shoulder contained a single protein (~11 kD) that was not a proteolytic fragment of HNF-1α.

Sequence data from the purified 11-kD protein, which we call DCoH, provided five peptide sequences ranging from 11 to 30 amino acids and containing a total of 95 amino acids. On the basis of this information, we isolated 20 individual complementary DNA (cDNA) clones encoding DCoH in an initial screen of 600,000 plaques from a rat liver cDNA library. The open reading frame of the longest insert, designated M1, encoded all five peptide sequences deduced from the purified DCoH protein and allowing the confirmation of 95 of the 104 predicted amino acids (Fig. 1). The M1 insert is comparable in size to the single mRNA identified by Northern (RNA) blot analysis of rat liver RNA with the M1 insert as a probe (*9*).

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Fig. 1. Predicted amino acid sequence of DCoH. HNF-1α purified from rat liver nuclear extracts (*8*) was denatured (5 M guanidine HCl, 10 mM DTT, for 1 hour at 37°C) and pyridinilated (20 mM 4-vinylpyridine for 15 minutes at 37°C) before being applied to a C8 reversed-phase HPLC column equilibrated with trifluoroacetic acid (TFA) (0.1 percent). Bound proteins were resolved with a linear gradient of acetonitrile (15 to 90 percent) in 0.1 percent TFA and then individually digested with sequencing grade trypsin or endoproteinase Asp-N (Boehringer Mannheim). Peptide fragments were separated on a C18 reversed-phase HPLC column in a linear gradient (0 to 90 percent) of acetonitrile in 0.1 percent TFA and sequenced in a pulse liquid sequencer (Applied Biosystems 477A). Degenerate oligonucleotides that encoded the ends of a 30–amino acid sequence of DCoH (double underlined), deduced from overlapping peptide fragments, were used as primers in a polymerase chain reaction (PCR) containing first-strand cDNA synthesized from poly(A)$^+$-selected rat liver RNA. A PCR product that correctly predicted the internal residues of the 30–amino acid sequence was used to screen random- and oligo(dT)–primed rat liver cDNA libraries (Stratagene). Clones were sequenced with Sequenase version 2.0 (U.S. Biochemical). The predicted amino acid sequence encoded by the open reading frame of the largest insert (M1) is shown. The nine amino acids not determined from the purified protein are underlined. The single difference in the predicted amino acid sequences of rat, mouse, and human DCoH is indicated. All nucleotide sequences have been submitted to GenBank (accession numbers, rat DCoH, M83740; mouse DCoH, M83741; human DCoH, M83742). Abbreviations for the amino acid residues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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M A G K A H R L S A E E R D Q L L P N L R A V G W N E L  28
                                                (mouse) V
E G R D A I F K Q F H F K D F N R A F G F M T R V A L Q  56

A E K L D H H P E W F N V Y N K V H I T L S T H E C A G  84

L S E R D I N L A S F I E Q V A V S M T * 104
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The M1 insert was used to screen mouse liver and human hepatoma cDNA libraries to obtain clones encoding murine and human DCoH. Although the longest human and murine cDNA clones only extend to amino acids 2 and 6, respectively, the deduced amino acid sequences of murine and human DCoH are similar to that of rat DCoH, with only a single conservative substitution (position 28) among the three predicted amino acid sequences (Fig. 1).

Stabilization of the HNF-1α dimer by DCoH. The predicted amino acid sequence of DCoH does not contain any known DNA binding motif, nor does it contain a cluster of basic residues that might mediate direct binding to DNA. Therefore, the fact that DCoH and HNF-1α from a rat liver nuclear extract copurify on a DNA affinity column suggests that DCoH interacts directly and stably with HNF-1α in vivo. In order to address this possibility, we tested whether antibodies to HNF-1α could coprecipitate DCoH after translation of the two proteins in vitro. Antibodies to HNF-1α precipitated DCoH only when HNF-1α was present in the reticulocyte lysate, and, as these experiments were performed in the absence of the HNF-1 DNA binding site, the interaction between these two proteins did not depend on HNF-1α binding to DNA (Fig. 2). We next examined a series of truncations and internal deletions of HNF-1α. Carboxyl-terminal deletions of HNF-1α, including one that removed the homeodomain, did not reduce binding of DCoH. However, removal of the amino-terminal 30 residues, which contain the dimerization domain of HNF-1α (*5*), eliminated the DCoH HNF-1α interaction. An internal deletion of the 35 amino acids immediately following the dimerization domain had little effect on DCoH binding to HNF-1α, indicating that the protein-protein interaction is not sensitive to conformational changes that might be caused by deletions near the amino terminus.

Because DCoH and HNF-1α were translated in the presence of [^{35}S]methionine, we could determine the relative stoichiometry of DCoH and HNF-1α in the precipitated complexes by quantitation of the radioactivity in the HNF-1α and DCoH protein bands after SDS-PAGE. Although the amount of precipitated HNF-1α varied, we observed a ratio of 1.1 ± 0.36 [mean ± standard deviation (SD)] DCoH molecules per molecule of HNF-1α (*10*). In that HNF-1α is a dimer (*5, 6*), we conclude that the precipitated complexes are heterotetramers that contain two molecules of HNF-1α and two molecules of DCoH. In the absence of HNF-1α, DCoH exists as a dimer (*11*), suggesting that the tetrameric complex is composed of a DCoH dimer bound to an HNF-1α dimer.

A homolog of HNF-1α, termed HNF-1β, shows striking conservation of the amino-terminal dimerization domain and homeodomain, but differs at the carboxyl-terminus (*6, 7, 12*). We tested DCoH for its potential to interact with HNF-1β. As for HNF-1α, mutants that lacked the dimerization domain of HNF-1β could not interact with DCoH (*13*). Carboxyl-terminal truncations of HNF-1β bound DCoH as well as the full-length protein, including one truncation that removed a charged region of HNF-1β that is similar in sequence to the POU-specific region of the POU homeodomain (*14*). This region has been implicated in protein-protein interactions between POU domain–containing proteins (*15*).

The observation that the DCoH–HNF-1α interaction depends on the dimerization domain of HNF-1α is consistent with the hypothesis that the stability of the native HNF-1α dimer is a result of the presence of a non–HNF-1α component of the native complex. As a means of testing whether DCoH can stabilize the HNF-1α dimer, we used a truncated form of HNF-1α that contained the first 428 amino acids of HNF-1α to compete with formation of the full-length HNF-1α dimer translated in vitro in the presence or absence of DCoH. The HNF-1α dimer translated in vitro is unstable (*5, 6*) and, upon challenge with truncated HNF-1α, the subunits of the dimers reassociated as homodimers of full-length HNF-1α, homodimers of truncated HNF-1α, and heterodimers of full-length and truncated HNF-1α (Fig. 3A, lane 3). The full-length homodimers, heterodimers, and truncated homodimers were pre-

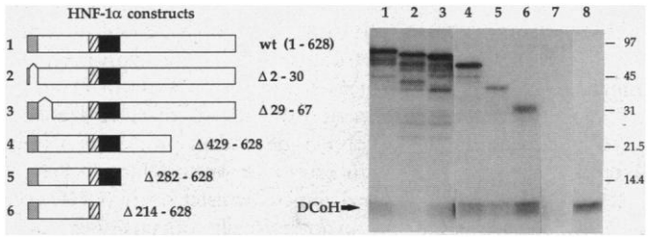

Fig. 2. DCoH directly interacts with HNF-1α. **Left**, HNF-1α mutants are depicted schematically with an indication of which amino acids have been deleted. [Δ, Deletion, followed by the position numbers of the deleted amino acid residues.] Construct 1 is wild-type (wt) HNF-1α. The dimerization domain (light shading at the amino termini), POU motif (slashes), and extended homeodomain (filled box) of HNF-1α are shown. **Right**, DCoH and the indicated HNF-1α mutants were cotranslated in rabbit reticulocyte lysate reactions that contained [^{35}S]methionine (*34*). HNF-1α and associated proteins were immunoprecipitated with antibodies to HNF-1α and then separated by PAGE under denaturing and reducing conditions on an acrylamide gel (14 percent). Radiolabeled proteins in the precipitated complexes were visualized by fluorography. Lanes 1 to 6, immunoprecipitated complexes from cotranslations containing DCoH and the indicated HNF-1α mutant; lane 7, immunoprecipitated complexes from a translation reaction containing DCoH alone. The migration position (arrow) of in vitro–translated DCoH (lane 8) is indicated, and the molecular size standards in kilodaltons are shown at the right of the gel.

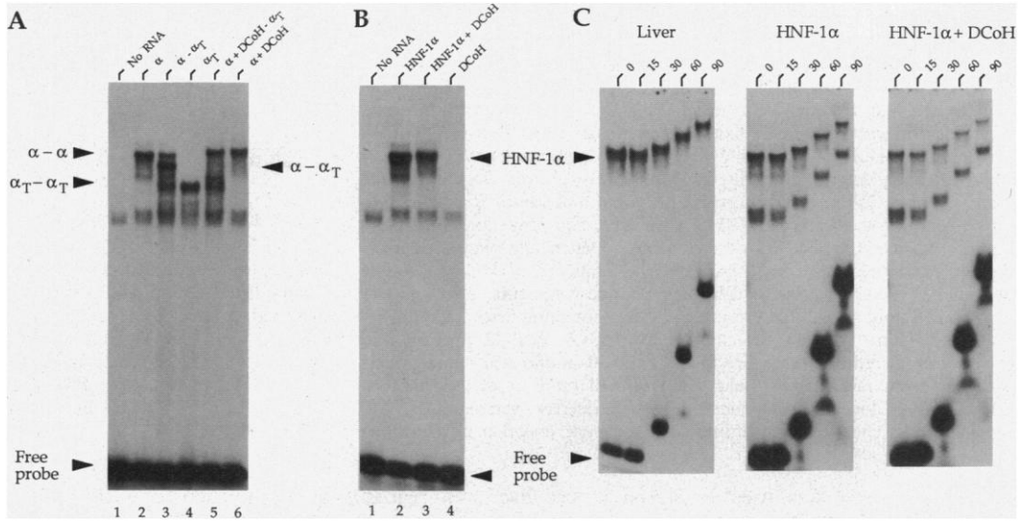

Fig. 3. DCoH stabilizes the HNF-1α dimer. (**A**) Translation reactions (*34*) primed with RNA for HNF-1α (α, lanes 2 and 3) or RNAs for HNF-1α and DCoH (α + DCoH, lanes 5 and 6) were mixed with an equal volume of a translation reaction primed with no RNA (lanes 2 and 6), or with RNA for a truncated form of HNF-1α that lacks the carboxyl-terminal 200 amino acids (α_T, lanes 3 and 5). The mixed reactions were incubated at room temperature (60 minutes) to allow the subunits of HNF-1α to reassociate. The ^{32}P-labeled β28 probe containing an HNF-1 binding site was then added to each sample and, after 30 min, the protein-DNA complexes were resolved by nondenaturing polyacrylamide gel electrophoresis (*6*). The migration position of homodimers of full-length HNF-1α (α-α), truncated HNF-1α (α_T-α_T), heterodimers of full-length and truncated HNF-1α (α-α_T), and free probe are indicated. The protein-DNA complex visible in the translation reaction that was not primed with RNA (lane 1) is a nonspecific complex that is present in all samples. Lane 3 contains a mixture of the translation reactions primed with RNA for α_T and with no RNA. (**B**) Inability of DCoH to bind to or to enhance HNF-1α binding to the HNF-1 site. The ^{32}P-labeled β28 probe was added to translation reactions primed with no RNA (lane 1), RNA for HNF-1α (lane 2), RNAs for HNF-1α and DCoH (lane 3), or RNA for DCoH (lane 4). Protein-DNA complexes were then resolved by electrophoresis on nondenaturing polyacrylamide gels. The migration positions of the HNF-1α–DNA complex and of the free probe are indicated. (**C**) Failure of DCoH to stabilize the HNF-1α–DNA complex. The ^{32}P-labeled β28 probe was added to rat liver nuclear extract (liver), or to translation reactions primed with RNA for HNF-1α alone (HNF-1α) or in addition to RNA for DCoH (HNF-1α + DCoH). After 30 minutes, an excess (100-fold) of unlabeled β28 probe sufficient to completely block the formation of HNF-1α–[^{32}P]DNA complexes (*8, 13*) was added to each sample. Portions of each reaction were loaded onto a nondenaturing polyacrylamide gel during the process of electrophoresis at the indicated time (minutes) after the addition of the unlabeled β28 probe. The migration positions of the HNF-1α–DNA complex and of the free probe are indicated for a sample loaded onto the gel immediately after the addition of the unlabeled probe.

sent with an apparent abundance of 1:2:1, indicating that these complexes are unrestricted in their ability to associate with each other (*16*). In contrast, the HNF-1α dimer translated in the presence of DCoH was stable, as indicated by the reduction in the number of heterodimers formed when the cotranslated HNF-1α–DCoH complex was mixed with truncated HNF-1α (Fig. 3A, lane 5). The slightly slower migration of the full-length HNF-1α homodimer translated in the presence of DCoH (Fig. 3A, lane 6), relative to that of HNF-1α translated alone (Fig. 3A, lane 2), probably reflects the presence of two molecules of DCoH bound to the HNF-1α dimer.

Cotranslation of HNF-1α and DCoH did not increase binding activity over that observed when HNF-1α was translated alone (Fig. 3B, lanes 2 and 3), and DCoH did not bind to the HNF-1α site by itself (Fig. 3B, lane 4). Similarly, the dissociation rate of HNF-1α from its recognition sequence, measured at room temperature, did not differ between native HNF-1α from liver nuclear extract and HNF-1α translated in vitro in the presence or absence of DCoH (Fig. 3C).

Enhancement of transcriptional activity of HNF-1α by DCoH. HNF-1α can activate transcription from HNF-1–dependent promoters when cotransfected into recipient cells that do not normally express HNF-1α, but the amount of activation varies according to the recipient cell used (*6, 7, 12, 17*). The observations that DCoH interacts directly with HNF-1α as part of the native HNF-1α complex and that DCoH can stabilize the native HNF-1α dimer suggest that the presence or absence of DCoH in a recipient cell might also affect the transcriptional activity of HNF-1α.

We tested the activity of HNF-1–dependent promoters cotransfected into recipient cells with expression vectors for HNF-1α or HNF-1α plus DCoH. We used chinese hamster ovary (CHO) cells as the recipients because they do not normally contain either HNF-1 DNA binding activity or DCoH mRNA (*13*). HNF-1α activated transcription of a gene with a fragment of the α-fibrinogen promoter (αFg-CAT), which contains a single HNF-1 binding site and binding sites for other factors in their in vivo contexts, fused to the chloramphenicol acetyltransferase (CAT) reporter gene (Fig. 4A). Cotransfection of increasing amounts of the DCoH expression vector along with the HNF-1α expression vector resulted in a dose-dependent increase in the HNF-1α–dependent CAT activity (Fig. 4A). DCoH similarly augmented the transcriptional activity of HNF-1α from a synthetic promoter that contained three HNF-1 binding sites (*18*). The DCoH expression vector, or the expression vector by itself, did not induce reporter gene activity (Fig. 4A), indicating that activation was HNF-1–dependent. We also found similar results for both promoters in COS cells (*18*).

On the basis of the amount of HNF-1 DNA binding activity detected in nuclear extracts of the transfected cells, we estimated that transfection of 5 μg of the HNF-1α expression vector yielded DNA binding activity that exceeded physiologic amounts. When a lower amount (10 ng) of the HNF-1α expression vector was used, we measured a 200-fold induction of HNF-1–dependent CAT activity in cells transfected with HNF-1α and DCoH relative to cells transfected with HNF-1α alone (Fig. 4B).

The HNF-1α protein contains a homeodomain and sequences related to the POU-specific region of POU domain–containing proteins (*19*) like GHF-1 (growth hormone factor–1, also called Pit-1). Therefore, we examined whether DCoH can augment the ability of this related factor (*20*) to activate transcription. The DCoH did not increase GHF-1/PIT-1–dependent CAT activity (Fig. 4C). In addition, DCoH did not substantially enhance the transcriptional activity of the glucocorticoid receptor, a potent transcriptional activator that belongs to the steroid hormone receptor superfamily (*21*) (Fig. 4C). The inability of DCoH to enhance the activity of these factors indicates that DCoH did not act by stabilization of the CAT message or protein and that it cannot function with all transcription factors.

We tested whether DCoH contained a domain that could activate transcription when fused to the DNA binding domain of the yeast

transcription factor GAL4. Full-length DCoH was linked to the amino-terminal end of the DNA binding and dimerization domains of GAL4 (*22*). This orientation was chosen because the amino-terminus of DCoH is more sensitive to modifications than is the carboxyl-terminus (*13*). The GAL4 DNA binding domain was chosen because it functions with a variety of heterologous transcription activation domains (*1*) and because it dimerizes, thus increasing the likelihood that DCoH would be presented in a dimeric configuration similar to that in the HNF-1α–DCoH complex.

The DCoH-GAL4 fusion protein did not activate transcription of a reporter construct driven by five tandemly arranged GAL4 binding sites (Fig. 5). A fusion protein that contained DCoH linked to the DNA binding domain of GAL4 and the transcriptional activation domain of the viral activator protein VP16 (*23*) exhibited a similar degree of activity in this system as did the GAL4-VP16 fusion protein alone (Fig. 5). Thus, the inability of DCoH-GAL4 to activate transcription was not a result of an inhibitory effect of DCoH. These results imply that DCoH cannot provide a transcriptional activation domain when fused to a DNA binding domain. We cannot eliminate the possibility that DCoH combines with some sequences within HNF-1α to generate an activation domain that would not be present in DCoH-GAL4.

Tissue-restricted expression of DCoH mRNA. Because DCoH stabilizes the HNF-1α dimer and activates HNF-1α–dependent transcription in vitro, these two proteins might be expected to be similarly regulated. We therefore used the ribonuclease protection assay to test for DCoH mRNA in adult murine organs. The mRNA for DCoH was most abundant in liver and kidney, was present at lower amounts in intestine and stomach, and at still lower amounts in lung, ovary, and brain (Fig. 6A). A labeled antisense riboprobe allowed detection of DCoH mRNA at low concentrations in rat cardiac tissue (*13*). Brain and heart tissues contained detectable DCoH mRNA, but did not contain the mRNA for either HNF-1α (*8, 17*) or HNF-1β (*6, 7, 12*). All tissues that contained mRNA for HNF-1α or HNF-1β contained the mRNA for DCoH. Furthermore, expression of DCoH mRNA was highest in the liver and kidney, the two tissues that contained the highest amount of HNF-1α protein in vivo (*8*).

The correlation between the expression of DCoH mRNA and HNF-1α and HNF-1β mRNAs was also maintained in the tissue culture lines studied (Fig. 6B). The mRNA for DCoH was abundant in well-differentiated hepatocyte cell lines, such as the murine Hepa1A and rat Fao cells, which express most of the hepatocyte-enriched gene products and HNF-1α binding activity (*6, 24*). The mRNA for DCoH was also expressed, albeit at lower amounts, in the C2 dedifferentiated hepatocyte cell line isolated from the Fao parent cell line (*25*) and in the FF5-1 somatic hybrids generated by fusing Fao cells with fibroblasts (*26*). Both C2 and FF5-1 express a limited number of hepatocyte-specific gene products and express HNF-1β instead of HNF-1α (*6, 24*). The mRNA for DCoH was also detectable in the poorly differentiated HTC hepatoma cell line, which does not express HNF-1 binding activity (*27*). Spontaneous revertants (Rev7 cells) isolated from the dedifferentiated C2 cells express most of the hepatocyte specific genes (*25*), including HNF-1α (*6, 8, 24*), and contained DCoH mRNA at a concentration that was intermediate between that found in the parent Fao cell line and the dedifferentiated C2 cell line.

Taken together, these results support the suggestion that HNF-1α requires DCoH to optimally activate transcription in a recipient cell.

Regulation of homeodomain function by interaction of HNF-1α and DCoH. A few examples exist in which the transcriptional activity of homeodomain-containing proteins is modulated. The herpes simplex virus protein VP16 specifically interacts with the second helix of the Oct-1 homeodomain (*28*). The interaction

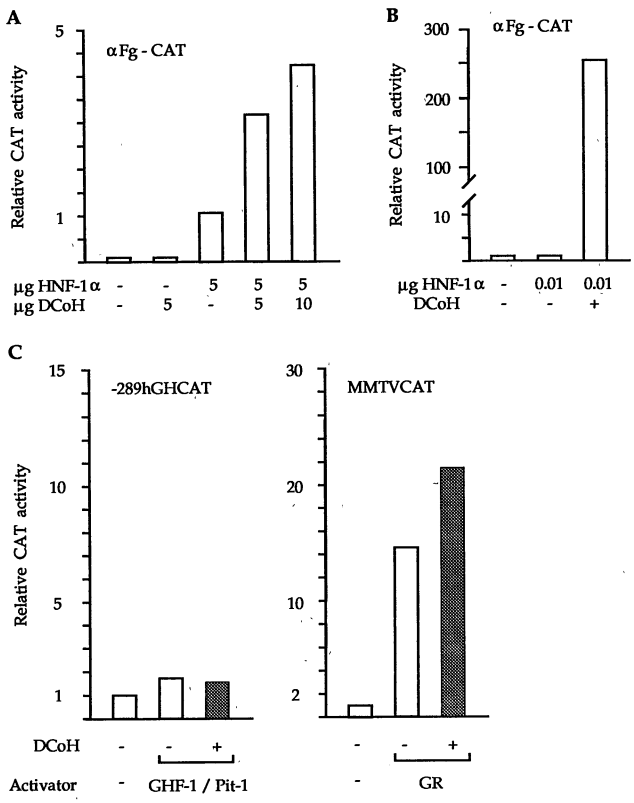

Fig. 4. Selective enhancement of the transcriptional activity of HNF-1α by DCoH. The CHO cells were transfected (*35*) with (**A**) the αFg-CAT reporter construct (5 μg) and the indicated amounts of the expression vectors encoding HNF-1α and DCoH; (**B**) the αFg-CAT reporter construct and 10 ng of the HNF-1α expression vector in the presence or absence of the DCoH expression vector (1 μg); or (**C**) the expression vector for the indicated activator protein and reporter construct either in the absence (open bars) or presence (shaded bars) of DCoH expression vector. Cells were assayed for CAT activity 36 to 48 hours after transfection. Results are presented as the averages of duplicate measurements in a representative experiment and are corrected for transfection efficiency (*35, 36*).

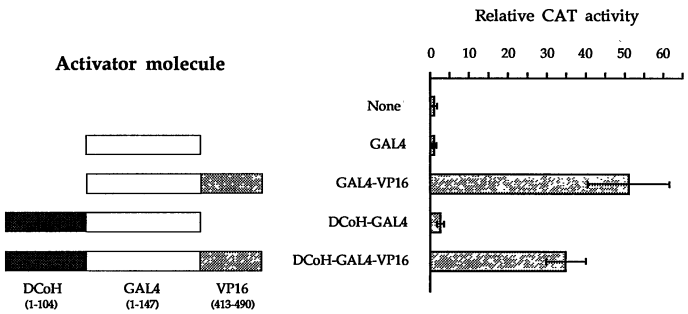

Fig. 5. Inability of DCoH to confer transcriptional activity to a DNA binding domain. The CHO cells were transfected (*35*) with the GAL_5 E1B-CAT reporter construct (15 μg) (*36*), which contains five tandemly linked GAL4 binding sites directing transcription of the CAT gene, and an expression vector (15 μg) encoding a fusion protein containing the GAL4 dimerization and DNA binding domains (*37*). The protein encoded by each of the expression vectors is shown schematically to the left, where the open box represents the dimerization and DNA binding domains of GAL4 (amino acids 1 to 147 of GAL4), the lightly shaded box at the carboxyl termini represents the 78–amino acid acidic activation domain of VP16 (amino acids 413 to 490 of VP16), and the darkly shaded box at the amino termini represents the entire 104–amino acid sequence of DCoH. Results are calculated and presented as indicated in the legend to Fig. 4.

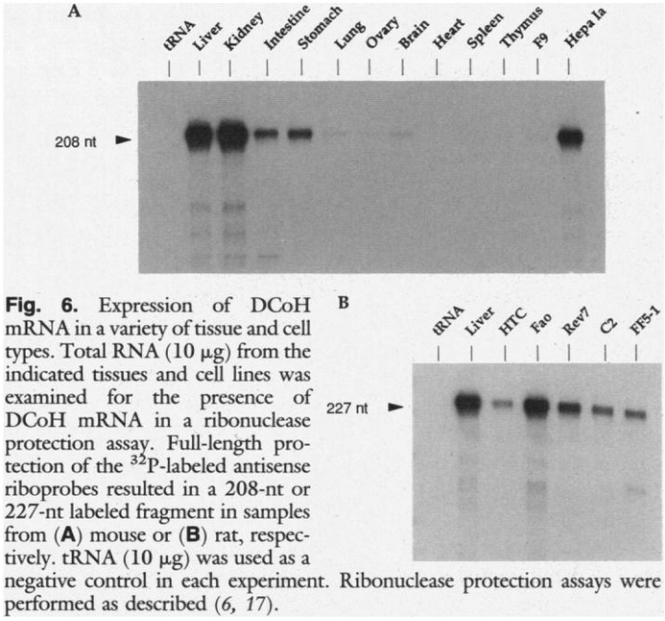

Fig. 6. Expression of DCoH mRNA in a variety of tissue and cell types. Total RNA (10 μg) from the indicated tissues and cell lines was examined for the presence of DCoH mRNA in a ribonuclease protection assay. Full-length protection of the ^{32}P-labeled antisense riboprobes resulted in a 208-nt or 227-nt labeled fragment in samples from (**A**) mouse or (**B**) rat, respectively. tRNA (10 μg) was used as a negative control in each experiment. Ribonuclease protection assays were performed as described (*6, 17*).

between these two proteins enhances the transcriptional activity of Oct-1 because VP16 provides Oct-1 with a potent acidic transcriptional activation region (*29*). The observation that DCoH cannot confer transcriptional activity to the GAL4 DNA binding domain indicates that it acts in a manner distinct from that of VP16.

It is also unlikely that DCoH serves as a coactivator or adaptor to facilitate the interaction between upstream activating transcription factors and the general transcription machinery (*30*). Unlike DCoH, which copurifies with HNF-1α, coactivators generally copurify with transcription factor II D (TFIID) (*30*), suggesting that this general transcription factor is the target of these molecules. Using coimmunoprecipitation, we found no evidence to suggest that DCoH facilitates an interaction between HNF-1α and TFIID (*31*). The conclusion that DCoH does not directly contact components of the general transcriptional machinery is also supported by the demonstration that production of large concentrations of DCoH in transfected cells did not squelch transcriptional activation by the glucocorticoid receptor.

We conclude that the ability of DCoH to enhance the transcriptional activity of HNF-1α is a result of its ability to stabilize the HNF-1α dimer. In this respect, DCoH acts in a manner similar to the adenovirus gene product E4, which enhances the transcriptional activity of E2F by stabilizing an E2F dimer (*32*). However, the demonstration that the E2F dimer binds more tightly to DNA in the presence of E4, whereas the DCoH-stabilized HNF-1α dimer does not, suggests that DCoH and E4 function by somewhat different mechanisms. We speculate that the association between DCoH and HNF-1α results in the appearance of an activation surface of HNF-1α that is not expressed when DCoH is absent. For example, if the activation domain of native HNF-1α is a composite surface formed by sequences from each of two subunits, the activation surface may be presented only when the dimer is stabilized by interaction with DCoH. This model is consistent with the observation that HNF-1α is a more potent transcriptional activator when a synthetic promoter contains multiple HNF-1 binding sites rather than a single site (*33*).

The broad tissue distribution of the DCoH mRNA suggests that there are other proteins whose activity is modulated by DCoH. Moreover, DCoH could be one of a group of cellular accessory proteins that regulate dimerization, thus providing a way to expand the regulatory activity associated with a limited number of proteins.

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9. D. Mendel and G. Crabtree, unpublished results. A Northern blot of total (10 μg) or poly (A)$^+$–selected (2.5 μg) rat liver RNA probed with the M1 insert, which was ^{32}P-labeled with random hexamers, identified a single species that migrates slightly faster than the 1.4-kb RNA marker.
10. The amount of ^{35}S associated with HNF-1α and DCoH in the precipitated complexes was determined by direct quantitation of the dried gel with an AMBIS radioactivity scanner (Ambis Systems, San Diego, CA). Background counts were subtracted to obtain the corrected counts. The relative stoichiometry of DCoH and HNF-1α in the precipitated complexes was determined from the corrected counts and the number of methionines in each construct as follows. The ratio of the number of molecules of DCoH to the number of molecules of HNF-1α is equal to the ratio of the corrected counts for DCoH divided by the number of methionines in DCoH to the corrected counts for HNF-1α divided by the number of methionines in the HNF-1α construct. Precipitated complexes containing wild-type HNF-1α and mutants 3 to 6 had an average of 1.1 DCoH molecules per HNF-1α molecule, with a range of 0.69 to 1.47. The precipitated complex containing mutant 2, which lacked the dimerization domain, did not contain DCoH beyond background values, as determined from the sample in Fig. 2, lane 7.
11. D. Mendel and G. Crabtree, unpublished results. Glutaraldehyde was added to bacterially produced DCoH in phosphate-buffered saline to produce a final concentration of 0.01 percent. After 5 minutes at room temperature, portions of the mixture were analyzed by polyacrylamide gel electrophoresis under denaturing and reducing conditions. In addition to the 11-kD DCoH band, a 26-kD protein band was observed in all samples that had been exposed to glutaraldehyde, but was absent from samples that did not have added glutaraldehyde. No other multimers were observed. These results indicate that DCoH exists as a dimer in the absence of HNF-1α.
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31. D. Mendel and G. Crabtree, unpublished results. HNF-1α and human TFIID were cotranslated with or without DCoH in rabbit reticulocyte lysates containing

[^{35}S]methionine to label the proteins. After translation, antibodies to HNF-1α were used to precipitate HNF-1α and associated proteins, and the precipitated proteins were resolved by polyacrylamide gel electrophoresis under denaturing and reducing conditions. Although a small amount of TFIID was present in the precipitated complexes, the amount of TFIID precipitated was not greater in the samples containing DCoH. We interpret these results as indicating that DCoH does not facilitate an interaction between HNF-1α and TFIID.

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34. The template that encoded wild-type HNF-1α was constructed by subcloning a 2174-bp Eco RI–Eco RV fragment of the murine HNF-1α cDNA (*17*) into pBluescript KS+ (Stratagene). RNA for full-length HNF-1α and the carboxyl-terminal deletions of HNF-1α were generated from this template linearized with a restriction enzyme. The template that encoded HNF-1α lacking the 30–amino acid dimerization domain (Δ 2-30) was constructed with a PCR method as described (*6*). The template that encoded HNF-1α lacking amino acids 29 to 67 was constructed with Stu I and Mlu I restriction sites within the HNF-1α sequence. Capped transcripts were generated by in vitro transcription in 30-μl reactions containing linearized plasmid (1 to 2 μg) and T7 RNA polymerase (30 to 40 units) as described (Boehringer Mannheim). Approximately one-tenth of the RNA from each reaction was then translated for 90 min at 30°C in a rabbit reticulocyte lysate translation reaction (25 μl) or, in the case of HBF alone (Fig. 2, lane 8), for 90 minutes at room temperature in a wheat germ lysate translation reaction (25 μl). Translation reactions were performed as indicated (Promega) in the presence of [^{35}S]methionine (20 μCi per reaction). Where indicated, translated proteins were immunoprecipitated with the addition of antiserum (1 μl) to HNF-1α (*6*) that had been adsorbed to Pansorbin (1.5 μl of slurry) (Calbiochem). After incubation (2 hours) at 4°C with occasional mixing, the Pansorbin that contained the antibody-protein complexes was centrifuged and washed twice in ice-cold phosphate-buffered saline (200 μl). The Pansorbin was then resuspended in SDS-PAGE sample buffer (25 μl) and heated for 3 minutes in a boiling water bath to elute the antibody-protein complexes.
35. The CHO cells (0.5 to 1 × 10^7) were transfected by electroporation with a Bio-Rad Gene Pulser set at 960 μF and 230 V. The total amount of DNA transfected into each sample of a given experiment was held constant by including the appropriate amount of pBJ5 expression vector lacking an insert, and the RSV-luciferase plasmid (2 μg) was included in each transfection as an internal control for transfection efficiency. The amount of expression vector and reporter construct cotransfected for the various activator proteins were pRSVrGHF1 (GHF-1), 20 μg, and 289hGHCAT, 5 μg; pSV2mRec (glucocorticoid receptor; GR), 15 μg, and MMTVCAT, 15 μg. Transfections of GR and GHF-1 included 15 μg and 5 μg of the DCoH expression vector, respectively, where indicated. Samples tested for glucocorticoid receptor activity were treated with dexamethasone (1 μM) for 8 hours prior to harvesting the cells. The CAT and luciferase activities were measured 36 to 48 hours after transfection as described (*6*). The murine HNF-1α expression vector constructed in the Srα-based pBJ5 expression vector has been described (*17*). The DCoH expression vector was constructed by inserting the M1 cDNA into the pBJ5 expression vector at a unique Eco RI site. The RSV-luciferase plasmid, the pRSVrGHF1 expression vector, the pRSVmRec mouse glucocorticoid receptor expression vector, and the $(\beta 28)_3$-CAT, −289hGHCAT, and MMTVCAT reporter constructs have been described (*36*). The αFg-CAT reporter construct consists of a 500-bp fragment of the α-fibrinogen promoter directing transcription of the CAT gene (*36*).
36. The HNF-1α expression vector and $(\beta 28)_3$-CAT reporter constructs have been described (*17*). The αFg-CAT reporter construct contains a contiguous 500-bp fragment of the α-fibrinogen promoter sequence, including the cap site, directing transcription of the CAT gene (J. P. Morgan and G. R. Crabtree, unpublished data). The pRSVrGHF1 expression vector and the −289hGHCAT reporter constructs have been described in L. E. Theill, J.-L. Castrillo, D. Wu, M. Karin, *Nature* **342**, 945 (1989) and C. Lefevre *et al.*, *EMBO J.* **6**, 971 (1987), respectively. The pRSVmRec glucocorticoid expression vector and the MMTV-CAT reporter construct have been described [M. Danielsen, J. P. Northrop, G. M. Ringold, *ibid.* **5**, 2513 (1986)]. The RSV-luciferase expression vector has been described (*8*). The pJL2 plasmid encoding GAL4-VP16 has been described [D. I. Chasman, J. Leatherwood, M. Carey, M. Ptashne, R. D. Kornberg, *Mol. Cell. Biol.* **9**, 4746 (1989)]. The $GAL4_5$E1bCAT reporter construct has been described [J. W. Lillie and M. R. Green, *Nature* **338**, 39 (1989)].
37. A construct encoding the full-length DCoH-GAL4-VP16 fusion protein was generated in pBluescript KS+ by a combination of overlap extension PCR (*38*) and subcloning procedures. The M1 insert, which encoded DCoH, and the pJL2 plasmid (*36*), which encoded amino acids 1 to 147 of GAL4 linked to amino acids 413 to 490 of VP16, were used as templates. All portions of the final construct contributed by PCR were sequenced. The predicted amino acid sequence at the DCoH-GAL4 junction of the fusion protein is VAVSMT-MKLLSS. Expression vectors for the various fusion proteins were generated by subcloning the appropriate segment of the DCoH-GAL4-VP16 construct into the pBJ5 expression vector plasmid (*6, 17*).
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