Hyperphenylalaninemia and Impaired Glucose Tolerance in Mice Lacking the Bifunctional DCoH Gene*

J. Henri Bayle, Filippo Randazzo, Georg Johnen, Seymou Kaufman, Andras Nagy, Janet Rossant, and Gerald R. Crabtree

From the Howard Hughes Medical Institute and the Departments of Developmental Biology and Pathology, Beckman Center for Molecular and Genetic Medicine, Stanford University, Stanford, California 94305, the Laboratory of Neurochemistry, National Institute for Mental Health, Bethesda, Maryland 20892, and the Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario M5G 1X5, Canada

The bifunctional protein DCoH (Dimerizing Cofactor for HNF1) acts as an enzyme in intermediary metabolism and as a binding partner of the HNF1 family of transcriptional activators. HNF1 proteins direct the expression of a variety of genes in the liver, kidney, pancreas, and gut and are critical to the regulation of glucose homeostasis. Mutations of the HNF1α gene underlie maturity onset diabetes of the young (MODY3) in humans. DCoH acts as a cofactor for HNF1 that stabilizes the dimeric HNF1 complex. DCoH also catalyzes the recycling of tetrahydrobiopterin, a cofactor of aromatic amino acid hydroxylases. To examine the roles of DCoH, a targeted deletion allele of the murine DCoH gene was created. Mice lacking DCoH are viable and fertile but display hyperphenylalaninemia and a predisposition to cataract formation. Surprisingly, HNF1 function in DCoH null mice is only slightly impaired, and mice are mildly glucose-intolerant in contrast to HNF1α null mice, which are diabetic. DCoH function as it pertains to HNF1 activity appears to be partially complemented by a newly identified homolog, DCoH2.

DCoH was discovered as a protein that copurified with the HNF1 family of transcription factors (1). The HNF1-DCoH complex directs the cell type-specific expression of a large group of genes in the liver, kidney, gut, and pancreas including α1-antitrypsin, phenylalanine hydroxylase (PAH), insulin-like growth factor I (IGF-I), α- and β-fibrinogen, and albumin (2–5). The HNF1 family includes two proteins, HNF1α and HNF1β, that can form homo- and heterodimers with each other and also heterotetramers with DCoH (1, 3, 6, 7).

HNF1α participates in a developmental cascade in which HNF4 directly regulates the expression of HNF1α, which in turn regulates a large group of downstream genes (8). Defects in this transcriptional cascade underlie maturity onset diabetes of the young (MODY3), an autosomal dominantly inherited syndrome characterized by early onset diabetes mellitus resulting from pancreatic β-cell dysfunction in response to glucose challenge (9–11). Mice lacking HNF1α due to targeted inactivation of its gene display hyperphenylalaninemia, Laron-type dwarfism, and a profound early onset diabetes mellitus as a result of the loss of expression of PAH and IGF-I as well as the loss of glucose-stimulated insulin secretion in response to hyperglycemia (12–14). Mice that are deficient in HNF1β expression do not support early embryogenesis because the visceral endoderm is not properly specified, and gastrulation is aberrant (15).

HNF1 proteins bind specifically to a pseudo-palindromic DNA target sequence as a dimer in a head-to-head arrangement (2, 3). Dimerization is directed by a 31-amino acid domain at the amino terminus of the protein that forms a unique antiparallel four-helix bundle when dimerized (16). The dimerization domain appears to be essential for HNF1 function because deletion of the dimerization domain reduces HNF1 DNA binding, and MODY3 mutations that result in amino acid substitution in this domain disrupt DCoH binding and reduce DNA binding (16, 17). This dimerization domain associates directly with a DCoH dimer. DCoH stabilizes the dimeric, DNA-binding form of HNF1 and augments HNF1-dependent transcription (1). Crystallographic structural studies have demonstrated that the α-helical HNF1 dimerization domain binds to an α-helical cap on the DCoH dimer that overlies a molecular saddle composed of antiparallel β-sheets (16, 18, 19). The DCoH saddle is similar in structure to the DNA binding saddle of the TATA-binding protein (20) and the RNA binding domain of the SRP9/14 domain of the signal recognition particle (21). These features have led to the hypothesis that DCoH may influence transcription by HNF1 by mechanisms other than its stabilization of HNF1 dimerization; however, no binding of DCoH to DNA or RNA has yet been demonstrated (22).

DCoH is a bifunctional molecule that was purified independently as a pterin 4α-carbinolamine dehydratase (PCD) (23, 24). This activity of cytoplasmic DCoH homotetramers catalyzes a step in the recycling of tetrahydrobiopterin (BH4), a cofactor for amino acid hydroxylases important for the catabolism of phenylalanine as well as the synthesis of catecholamines, serotonin, and nitric oxide (25). DCoH specifically dehydrates the 4α-OH-BH4 product of hydroxylases to the quinonoid form of BH4 before it can spontaneously rearrange to the 7R isomer, which acts as an inhibitor for PAH (26, 27). As such, DCoH can enhance the activity of PAH and has been called the PAH...
stimulating protein (PHS). Rare cases of hyperphenylalaninemia in humans are caused by DCoH mutations (27, 28), and the progressive pigmentation disorder vitiligo has been correlated with loss of DCoH enzymatic activity apparently without mutation or hyperphenylalaninemia (29).

To define further the roles of DCoH, we have created mice in which the DCoH gene is deleted. Although DCoH null mice are viable and fertile, they have hyperphenylalaninemia and a predisposition to cataract formation. Surprisingly, HNF1 function is only partially impaired in DCoH null mice. Although cytoplasmic DCoH expression is undetectable in liver and kidney extracts, a complementing activity that interacts with HNF1 is observed in DCoH null nuclear extracts. This activity is likely to be the result of the low level nuclear expression of a second DCoH-related gene, DCoH2.

EXPERIMENTAL PROCEDURES

Preparation of DCoH Knockout Mice—The murine DCoH genomic locus was isolated from a λ genomic library of strain 129 mouse DNA. A 2.9-kb BanI fragment containing sequences 3′ to the DCoH gene was inserted between the herpes simplex virus thymidine kinase gene and the neomycin resistance gene (neor) in the vector pKS(NT). Sequences 5′ to the DCoH gene including exon 1 were derived from a 2.8-kb NcoI fragment and were inserted 5′ to the neor gene. The resulting knockout construct pKS(NT)/DCoH2 was electroporated in R1 ES cells. Neomycin- and ganciclovir-resistant colonies were screened by RNA was transferred to GeneScreen Plus filters (PerkinElmer Life Sciences), cross-linked, prehybridized for 1 h in Church buffer (0.5 M Na-HEPES, pH 7.6, 150 mM NaCl, 0.5 mM EDTA, 5% glycerol, and 1.25 ug of poly(dI-dC) incubated on ice for 1 h. Supershifting antibodies, if used, were added after 30 min of binding. Reactions were run on a 5% acrylamide gel buffered with 0.5 X TBE. The experiment displayed in Fig. 4 utilized an oligonucleotide derived from the rat β-fibrinogen promoter. Antiserum used in gel shift experiments was a mouse anti-rat DCoH polyclonal antiserum that recognizes DCoH in a native conformation but does not appear to react to DCoH2.

Transfections—Cells were transfected by electroporation at 230V, 980 microfarads (Chinese hamster ovary) and 250 V, 960 microfarads (COS) with a Bio-Rad gene pulser. DCoH and mouse DCoH2 were cloned in the expression vector DF30 with an amino-terminal FLAG epitope tag. Expression was driven by the SRα promoter.

Northern Blot—RNA from liver was prepared by the acid-phenol method (32) by quickly mincing 0.5 g of mouse liver on ice and transferring to 2 ml of solution D (32) in a 15-ml Falcon 2059 tube and homogenizing for 5 s with a Branson homogenizer. All other steps were performed exactly as described. RNA (10 ug) was resolved on a 1.3% formaldehyde/agarose gel in 1 X MOPS buffer (20 mM MOPS, 5 mM sodium acetate, 1 mM EDTA) with 1.1% formaldehyde.

RNA was transferred to GeneScreen Plus filters (PerkinElmer Life Sciences), cross-linked, prehybridized for 1 h in Church buffer (0.5 M sodium phosphate, pH 7.0, 7% SDS, 1% bovine serum albumin) at 65 °C and hybridized in Church buffer >12 h with 107 cpm of probe. Filters were washed three times with 1 X SSC at 65 °C. Probes were prepared by the random priming method (33). Results were quantitated by spot densitometry and normalized relative to glyceraldehyde phosphate dehydrogenase levels.

RESULTS

Generation of DCoH Null Mice—A null allele in the DCoH gene was generated in ES cells, and DCoH null mice were created. The DCoH gene is composed of four exons with the first exon containing 5′-noncoding sequences and the initiation codon. The final three exons include the remaining coding and 3′-noncoding sequences and are contained entirely in 6 kb of murine chromosome 10 (Fig. 1 A; see “Experimental Procedures”). 7.5 kb including exons 2, 3, and 4 of the DCoH gene were replaced by the neor gene driven by the phosphoglycerate kinase promoter in the knockout construct pKS(NT)/DCoH2. Homologous recombination in ES cells resulted in the deletion of all but one exon from the DCoH gene.

Mice were derived from two targeted ES cell clones, and no dominant phenotypes were observed. Heterozygous animals were intercrossed to generate DCoH knockout mice. DCoH null mice are viable, fertile, and thrive for longer than 1 year. Mice containing the DCoH null allele are born in the expected ratios from heterozygous crosses. To demonstrate that DCoH expression was impaired by the recombinant allele, DCoH RNA expression in the liver was examined by Northern blot in a representative litter of a heterozygous mating (Fig. 1D with the genotyping using allele-specific PCR displayed in Fig. 1C). DCoH RNA expression was reduced in heterozygous animals and was absent in null animals. Western analysis using rabbit polyclonal antiserum directed to DCoH revealed that DCoH expression in the liver, kidney, and eye was severely attenuated in mice homozygous for the knockout allele (Fig. 1E).

Hyperphenylalaninemia in DCoH Null Mice—The PCD activity of DCoH catalyzes a step in the recycling of BH4, the cofactor of aromatic amino acid hydroxylases. Dehydratase activity was absent in extracts derived from DCoH mouse liver, and the by-product 7-hippurin accumulated in the mice (Table I). Hyperphenylalaninemia resulting from inhibited PAH activity in vivo was fully penetrant in DCoH/PCD/PHS null mice.
but variable in its expressivity and was generally below the levels measured in cases of classic phenylketonuria (in which PAH is mutated). Although detailed tests of cognitive skill have not been performed on DCoH mice, hyperphenylalaninemia does not appear to have seriously affected their motor function, feeding, or pup rearing. No obvious phenotypes indicative of maternal phenylketonuria were observed in pups derived from DCoH null mothers even if the pups were null for DCoH themselves. Serious elevation of alkaline phosphatase or glutamate transaminase activity in serum as evidence of liver degeneration was not observed in the null mice (Table I). DCoH mice frequently exhibit a mild hypopigmentation relative to their wild-type littermates when crossed onto the non-Agouti c57BL/6J genetic background. Infrequently (<10%), an unpigmented spot is observed on the belly of DCoH mice.

Glucose Metabolism in DCoH Null Mice—In humans, mutation in the HNF1α gene gives rise to MODY3, and HNF1α null mice have pronounced hyperglycemia and glucosuria associated with diabetes mellitus. No evidence of fasting hyperglycemia was observed in DCoH mice between the ages of 16 and 30 weeks (Fig. 2A and data not shown). A more rigorous test for diabetes is the glucose tolerance test in which diabetic animals

| Plasma [PHE] | PHS | PAH | 6-Biopterin | 7-Biopterin | Serum ALP | Liver ALP | GPT | Albumin |
|-------------|-----|-----|-------------|-------------|-----------|----------|-----|----------|
| DCoH+/−     | 76  | 23.8 (8) | 0.68 (2)  | 21.9 (2)    | ND        | ND       | 49.2 (2) | 0.137 (10) |
| DCoH−/−     | 76  | 23.8 (8) | 0.68 (2)  | 21.9 (2)    | ND        | ND       | 49.2 (2) | 0.137 (10) |
| DCoH−/−     | 357 | 7 (2)    | 0.92 (2)  | 7 (2)       | ND        | ND       | 49.2 (2) | 0.137 (10) |
| DCoH−/−     | 357 | 7 (2)    | 0.92 (2)  | 7 (2)       | ND        | ND       | 49.2 (2) | 0.137 (10) |

FIG. 1. Targeted deletion of the murine DCoH gene. A, schematic representation of the murine DCoH gene (upper), the targeting construct, and the deletion allele of DCoH after homologous recombination into the genome of R1 ES cells. 7.5 kb of DNA containing the entire coding sequences for DCoH with the exception of the initiating methionine was replaced with the neo' gene driven by the phosphoglycerate kinase promoter. The herpes simplex virus thymidine kinase gene flanks the 3'-arm to select against untargeted insertion events with ganciclovir. B, targeted insertion in ES cell clone 2–16 was confirmed by Southern blotting of a HindIII digest (left) or BglII/EcoRI double digest (right) of genomic DNA detected with the 5'-outside probe. C, PCR genotyping of the adult progeny of an intercross of DCoH heterozygous parents. The primer sequences giving the lower product are deleted in the targeted allele, whereas the targeted allele is detected with primers annealing to the neo' gene. D, analysis of DCoH RNA expression in liver of DCoH wild-type, heterozygous, or null mice. The genotype is indicated above and in C. A Northern blot of total liver RNA was probed with a 312-bp cDNA containing the entire DCoH coding sequence. E, Western blot of total cellular lysate or nuclear extract (NE) from DCoH wild-type or null mice from the indicated tissues. Denatured protein samples were resolved by SDS-PAGE and DCoH was detected with rabbit α-DCoH serum.
typically display an increased peak in the blood glucose level after challenge and require a longer period to clear blood glucose into muscle, fat and liver. DCoH null mice were found to be mildly glucose-intolerant \((n/11005/28)\) relative to their heterozygous and wild-type controls \((n/11005/25)\). Glucose levels 1 h after challenge were elevated to an average of 50 mg/dl higher than in control animals, and glucose levels remained elevated 2 h after challenge. Impaired glucose intolerance (measured as a value of \(11022/200\) mg/dl at 1 h postchallenge) was of incomplete penetrance as the response to glucose was abnormal in about half of the null mice when on the outbred CD1 background, whereas only 10% of control mice were glucose-intolerant. Non-specific ketonuria detectable in DCoH \(/-/11002/-/11002\) mice (data not shown) was most likely the result of phenylpyruvate secondary to hyperphenylalaninemia rather than diabetes because \(\beta\)-hydroxybutyrate levels are low in null mice (Table I). DCoH null mice were found to be more glucose-tolerant in general when crossed for three generations on the inbred c57BL/6J genetic background (data not shown). Interestingly, insulin levels were somewhat elevated in fasted DCoH mice and did not rise to the same degree as wild-type mice after glucose challenge (Fig. 2B).

Defects in insulin secretion after glucose challenge have been observed in pancreatic islets isolated from HNF1α mice (13, 34). Eye Abnormalities in DCoH Null Mice—DCoH expression has been detected in the eye (Fig. 1E), and this expression appears to be localized primarily to the retinal pigmented epithelium (35). Interestingly, DCoH mice were found to have a predisposition to the development of ocular abnormalities including slight microphthalmia and cataract formation. Lens opacities were visually detectable in 18.2% of DCoH null mice maintained on the outbred CD1 genetic background, and the predisposition is recessive in inheritance; no cataracts were observed in the heterozygous progeny of affected parents \((n/11005/25)\). Cataracts typically were initiated in the posterior cortex of the lens displaying characteristic Morgagnian granules of degenerated lens fibers (Fig. 3C) and could mature to cover the entire lens. The age of first detection varied widely with the earliest detection at 12 days soon after the eyelids open up to an age of 1 year, although most affected animals presented by the age of 24 weeks (Fig. 3B). Cataracts were observed in both independent lines of DCoH null mice and were most commonly unilateral. Interestingly, the incidence of cataract formation was reduced in the c57BL/6J inbred genetic background despite the inherent predisposition to eye abnormalities in this strain of mice. These findings indicate that other genetic factors in the CD1/129 background contribute with the loss of DCoH to initiate an opacity in the lens. The penetrance of nearly one-fourth of DCoH null mice suggests that such a factor may be a recessive allele of a single gene giving a synthetic phenotype with DCoH nullizygosity. Confounding this hypothesis is the observation that progeny of parents that each have cataracts did not display an increased penetrance of the phenotype.

Cataract formation is common in human subjects with type 2

\[\text{FIG. 2. Impaired glucose tolerance in DCoH null mice.} \]

Glucose levels from mice fasted overnight (12–14 h) were determined before and 1 h after a bolus (2 g/kg, intraperitoneal injection) of glucose. Results are represented as the average of a pool of DCoH wild-type and heterozygous animals denoted \(+/11001/11001\) \((n/11005/25)\) or from DCoH null mice \((n/11005/28)\). Error bars indicate ± 1 S.D. The difference in the glucose level between the population was judged to be significantly different with a probability of greater than 0.995 by Student’s \(t\) test. \(B\), plasma insulin levels (μg/ml) before and after glucose challenge of fasted mice. \(p\) values were determined by Student’s \(t\) test.

\[\text{FIG. 3. Development of cataract in DCoH null mice.} \]

A, DCoH null mouse showing unilateral lens opacity. \(B\), plot displaying the frequency of first visual observation of cataract in DCoH null mice in age intervals up to 1 year. \(C\), section of a DCoH null mouse with posterior cortical cataract (right) and a DCoH heterozygous mouse of similar age with a healthy eye (left). Transverse sections of paraffin-embedded tissue were stained with hematoxylin and eosin. Note the vacuoles visible in the iris and the Morgagnian granules of degraded and fluid filled lens tissue underlying the posterior capsule.
diabetes with the underlying cause thought to be an osmotic inflow into the lens resulting from increases in the lenticular concentration of sorbitol, a product of increased glucose metabolism or glycation of lens proteins by hyperglycemia. Cataract formation in murine models for diabetes is not commonly observed. No correlation between glucose intolerance and the incidence of cataract could be made in DCoH null mice; mice that consistently failed the glucose tolerance test did not necessarily develop cataracts, and mice that had cataracts were not necessarily glucose-intolerant. It is therefore unlikely that the cataracts observed in DCoH null mice are a secondary effect of diabetes mellitus because overt hyperglycemia was not observed in these mice.

**HNF1 Activity in DCoH Null Mice**—Although the cytoplasmic PAH-stimulating protein/PCD activity of DCoH was defective and DCoH null mice have hyperphenylalaninemia, the phenotype of DCoH null mice is not similar to null mutations in the HNF1a or β genes, as development is largely normal, and the mice are only mildly glucose-intolerant rather than overtly diabetic. To examine more clearly the nuclear function of DCoH, HNF1o biochemical activity in the liver was examined. Nuclear extracts were prepared from livers of DCoH null mice and wild-type mice, and electrophoretic gel mobility shift assays of a radiolabeled HNF1-specific oligonucleotide were performed (Fig. 4). Liver nuclear extracts derived from both wild-type and mutant animals contained similar levels of a DNA binding activity that could be specifically blocked by competition with an HNF1 binding site but not by an oligonucleotide of unrelated sequence. HNF1o levels were also found to be similar in both DCoH null and wild-type nuclear extracts by Western blotting (data not shown). The finding that HNF1 DNA binding activity *in vitro* was not dramatically affected by loss of DCoH in liver nuclear extracts was consistent with the known capacity of HNF1, when overexpressed by transfection or synthesized *in vitro*, to bind to DNA without DCoH. To examine the role of DCoH in HNF1-dependent transcriptional activity as well as DNA binding activity, transcription reactions were performed *in vitro* using nuclear extracts prepared from wild-type and DCoH null mice. No defects in transcription from the HNF1-dependent albumin promoter were detected (data not shown). These studies were extended by examination of the expression of several genes having HNF1 DNA binding sites in their regulatory regions (Fig. 5). RNA levels of several HNF1 target genes including the α1-antitrypsin gene, albumin, and PAH were reduced in DCoH mutant mice. The defect in expression of HNF1 targets was small (frequently less than 2-fold) but consistent between experiments with different mice. In some cases, such as the albumin and α1-antitrypsin genes, the relatively subtle defect in RNA expression resembles that observed in HNF1a mutant mice. However, in HNF1a null mice a strong loss in PAH and IGF-I expression has been reported (12, 14) whereas only a 2-fold loss in PAH levels is observed in DCoH null mice, and no defect in IGF-I expression has been observed. The reduced level of PAH expression is consistent with the reduced level of PAH activity observed in livers of DCoH null mice (Table 1).

**Detection of a Complementing Activity in DCoH Null Nuclear Extracts**—The observation that HNF1-dependent transcription is only subtly diminished by the deletion of DCoH leads to several possible conclusions: DCoH binding to HNF1 has a marginal effect on HNF1-dependent transcription, DCoH activity is necessary only in a subset of HNF1-dependent promoters, or the ability of DCoH to form a complex with HNF1 is complemented by other factors. Perhaps the best defined biochemical activity for DCoH function in HNF1 activity is the capacity of DCoH to stabilize the dimeric form of HNF1.

**Fig. 4. HNF1 DNA binding activity is not impaired in nuclear extracts from DCoH null mice.** An electrophoretic mobility shift assay was performed using nuclear extracts derived from liver of DCoH wild-type or DCoH null mice and a 32P-labeled oligonucleotide containing the HNF1 binding site from the rat β-fibrinogen promoter. The addition of a 50-fold excess of an unlabeled oligonucleotide containing an HNF1 binding site (HNF1 oligo) but not an unrelated site (Oct1 oligo) could block DNA binding activity in each extract by competition. HNF1 DNA binding activity could be supershifted by inclusion of a mouse polyclonal antisera to a native conformation of DCoH (α DCoH) with the nuclear extract from DCoH wild-type mice, but not DCoH null mice.
a complex with HNF1 is detectable in both whole cell and nuclear extract. Because the DCoH knockout allele deleted all but one amino acid of the coding region of the gene, it seemed unlikely that the presence of α-DCoH immunoactivity in null liver nuclear extracts was caused by a partial knockout, but rather by the presence of a close homolog to DCoH. Direct searches through the EST (expressed sequence tag) libraries from mouse and human using the BLAST algorithm (36) and the coding sequences of DCoH initially failed to detect such a homolog; however, an alternate search using only sequences from exon 3 of DCoH (which encodes the recognition helix for HNF1 binding and DCoH tetramerization) revealed human genomic sequences from chromosome 5 with extensive similarity when decodified to DCoH. Probing these genomic sequences through the EST libraries (37) revealed the presence of a cDNA encoding a close DCoH homolog, DCoH2 (Fig. 7A), expressed in libraries derived from several tissues of mouse and human including liver and kidney. DCoH2 shares 68% amino acid identity and 86% similarity with DCoH. The DCoH2 cDNA was cloned into a mammalian expression vector, expressed in COS7 cells, and lysates from these cells were found to contain anti-DCoH cross-reacting activity in Western blots (Fig. 7C) similar to that observed in liver nuclear extracts derived from DCoH null mice. The expression of DCoH2 appears to be strongest in the intestine especially in relation to the liver and kidney (Fig. 7D).

The crystal structure of DCoH has been determined (18, 19), and it was therefore of interest to map the regions of identity and dissimilarity between DCoH and DCoH2 (Fig. 7B). The regions of greatest dissimilarity between the two proteins are in the first six amino acids that are not resolved in the crystal structure, at the tip of the stirrups of the saddle, and along helix 3 in side chains that point into the solvent. Not surprisingly, the hydrophobic core of the protein including the amino acids that direct dimerization are strongly conserved between the two proteins. Significantly, helix 2, which comprises the top of the hat of the DCoH dimer and directs tetramerization with another DCoH dimer or with HNF1 (16), has a nearly identical amino acid composition between DCoH and DCoH2. This similarity at the interface of tetramerization is likely to conserve the ability of DCoH2 to interact with HNF1 and underlie the complementation of DCoH loss toward HNF1 activity.

**Fig. 5. Expression of HNF target genes in DCoH null mice.** A Northern blot of RNA prepared from liver of 14-week-old littermates is presented with DCoH genotypes indicated at the bottom. Gene expression was probed for alcohol dehydrogenase 1 (ADH 1), albumin, PAH, IGF-1, α1-antitrypsin, DCoH, and glyceraldehyde phosphate dehydrogenase (GAPDH). Numbers represent the percentage expression level relative to the wild-type sample in lane 2, normalized to relative glyceraldehyde phosphate dehydrogenase levels.

**Fig. 6. DCoH binding to HNF1 is complemented by a related activity.** A, HNF1 dimers are stable in nuclear extracts derived from DCoH null mice. Left panel, nuclear extracts were prepared from Chinese hamster ovary cells transfected with HNF1α (ZNF1) or with both HNF1 and DCoH (HNF1/DCoH). An electrophoretic mobility shift assay was performed with a labeled oligonucleotide derived from the mouse albumin promoter and the nuclear extract or with 15 ng of a purified HNF1α species (HNF1αr) truncated at amino acid 280. Inclusion of HNF1αr with HNF1 nuclear extract promotes the formation of mixed dimers of HNF1-HNF1αr that migrate intermediately between HNF1α and HNF1αr dimers alone. The exchange of dimers of HNF1α with HNF1αr is inhibited by DCoH (fourth lane). Liver nuclear extract derived from wild-type (middle panel) or DCoH null mice (right panel) was incubated with 50 ng of purified DCoH, 15 ng of purified HNF1αr, or with both along with labeled HNF1 binding site. HNF1 dimers from each extract source were resistant to dimer exchange with HNF1αr. HNF1 DNA binding activity from nuclear extracts could be supershifted with antiserum to HNF1α (αHNF1α) which recognizes a carboxyl-terminal epitope not present in HNF1αr. B, identification of a DCoH-related protein in the nucleus. Upper panel, Western blot of the indicated amounts of liver nuclear extract derived from DCoH null or wild-type mice separated by SDS-PAGE and probed with rabbit α-DCoH antiserum. A cross-reacting activity is detectable in the null nuclear extract. Lower panel, native gel Western blot of total liver lysate (Total) and liver nuclear extract (Nuclear) derived from wild-type mice (+/+ ) and DCoH null mice (−/−). Nuclear DCoH bound to HNF1 migrates slowly in the native gel, whereas the large cytoplasmic pool of DCoH homotetramers migrates quickly. The cross-reacting activity in DCoH null liver appears to be present primarily with HNF1 because it comigrates only with the slow form of DCoH.
poor maternal behavior, and hypopigmentation (38). These ob-
in PAH resemble classic phenylketonuria including ataxia,
ing DCoH enzymatic activity (27, 28) whereas strong mutants
and in human subjects with homozygous mutations inactivat-
the observed degree of hyperphenylalaninemia resembles that
in the nuclear function of DCoH. The nuclear
activity is expressed (44). These findings have raised the hy-
derm even if a mutant form of DCoH defective in enzymatic
injection of DCoH RNA into early
is largely cytoplasmic until the onset of transcription at the
midblastula transition when the protein translocates to the
has been observed in these tissues which do not express detect-
to melanin and neurotransmitters, nuclear staining for DCoH
similar to those observed with the loss of HNF1 activity. Al-
was surprising that DCoH null mice did not exhibit phenotypes
hypothesis that DCoH may have nuclear activities other than
expression in combination with DCoH. The complementation of
mice, a complete analysis of the role of DCoH in HNF1-directed
Because HNF1 activity is only partly impaired in DCoH null
sion in the eye has not been observed. Although dehydratase
activity may contribute to the synthesis of melanin in the
retinal pigmented epithelium, the mechanism of lens fiber dis-
ruption when DCoH expression is lost is not clear. No correla-
bin 3 (data not shown) were found to be reduced to a degree
antitrypsin, albumin (Fig. 5), and \( \beta \)-fibrinogen and antithrom-
in rods DCoH is expressed abundantly in liver and kidney as well as in the
gut, stomach, and pancreas, all tissues that express HNF1 (1).
DCoH expression has also been observed in skin, brain, adrenal
gland, and the eye (35, 40–43). Although these tissues likely
use the enzymatic activity as part of the synthesis of precursors
to melanin and neurotransmitters, nuclear staining for DCoH
has been observed in these tissues which do not express detect-
able HNF1. During early frog development, DCoH expression
is largely cytoplasmic until the onset of transcription at the
midblastula transition when the protein translocates to the
nucleus in the absence of HNF1 expression (35). Very interest-
ingly, injection of DCoH RNA into early Xenopus embryos
results in ectopic hyperpigmentation in the developing ecto-
derm even if a mutant form of DCoH defective in enzymatic
activity is expressed (44). These findings have raised the hy-
thesis that DCoH may have nuclear activities other than
interaction with HNF1.

Because DCoH enzymatic activity was severely reduced, it
was surprising that DCoH null mice did not exhibit phenotypes
similar to those observed with the loss of HNF1 activity. Al-
though DCoH stabilizes the DNA binding dimeric form of
HNF1 and augments HNF1-dependent transcription in trans-
fection assays with reporter genes (1), the necessity of cofactor
binding in the natural state was unknown. Expression of \( \alpha \)-
antitrypsin, albumin (Fig. 5), and \( \beta \)-fibrinogen and antithrom-
bin 3 (data not shown) were found to be reduced to a degree
similar to that found in HNF1\(_{\alpha}\) null mice. However, PAH RNA
levels and activity (Table 1), although reduced, were not atten-
duated to the degree observed in HNF1\(_{\alpha}\) null animals, which
have a strong hyperphenylalaninemia not caused by the loss of
BH4 recycling, but because PAH transcription is disrupted (12).
Similarly, the strong defect in IG-F-I transcription observed in
HNF1\(_{\alpha}\) null mice was not mimicked in DCoH null mice (14).
Because HNF1 activity is only partly impaired in DCoH null
mice, a complete analysis of the role of DCoH in HNF1-directed
transcription will likely require the disruption of DCoH2 ex-
pression in combination with DCoH. The complementation of
DCoH transcriptional function by DCoH2 makes it likely that
DCoH will not be a common target for mutation in MODY

**DISCUSSION**

The DCoH protein is remarkable in that it performs separate
cytoplasmic and nuclear functions that affect metabolism and
gene expression. Loss of DCoH expression results in a substan-
tial loss in the cytoplasmic, enzymatic activity of DCoH, but an
incomplete loss in the nuclear function of DCoH. The nuclear
activity of DCoH is likely to be complemented by a close gene
family member, DCoH2, reported here. Although apparently
dispensable for normal embryonic and postnatal development,
DCoH functions in several physiological roles, and its loss in
mice results in an increased frequency of cataract formation,
hyperphenylalaninemia, and impaired glucose tolerance.

The loss of DCoH enzymatic activity un couples BH4 recy-
cling from PAH activity and results in hyperphenylalaninemia.
The observed degree of hyperphenylalaninemia resembles that
of weak mutant alleles of murine GTP cyclohydrolase and PAH
in human subjects with homozygous mutations inactivating
DCoH enzymatic activity (27, 28) whereas strong mutants
in PAH resemble classic phenylketonuria including ataxia,
poor maternal behavior, and hypopigmentation (38). These ob-
serations suggest that there is an incomplete loss of PAH
activity in the absence of DCoH that may be the result of
partial complementation by DCoH2 even though levels of
DCoH2 are very low relative to those of DCoH itself and appear
to be confined to the nuclear compartment except perhaps in the
gut where DCoH2 appears to be more abundant.

DCoH null mice commonly developed cataracts, most fre-
cently unilateral, but occasionally in both eyes. Although
cataracts are common in aging humans and mice, cataract
formation was observed at ages varying from before weaning to
up to 1 year, but most commonly after 16 weeks of age. DCoH
is expressed in the eye and appears to be confined to the retinal
pigmented epithelium and not the lens whereas HNF1 expres-
sion in the eye has not been observed. Although dehydratase
activity may contribute to the synthesis of melanin in the
retinal pigmented epithelium, the mechanism of lens fiber dis-
ruption when DCoH expression is lost is not clear. No correla-

**Fig. 7. Identification of DCoH2, a close homolog of DCoH.** A, sequence similarity between DCoH and DCoH2. Alignment of the
amino acid sequences of DCoH and DCoH2 is in one-letter code. Iden-
tical amino acids are shaded with red, and similar amino acids as
defined by the BLAST algorithm are shaded in green. Blue indicates
strongly divergent amino acid sequence. The proteins are identical in
68% of their amino acids with 85% similarity. B, structural similarity
between DCoH and DCoH2. The crystal structure of a dimer of DCoH is
displayed using Insight II with amino acid residues that are identical or
similar between DCoH and DCoH2 colored red and strongly dissimilar
residues as shown in A colored blue. C, rabbit \( \alpha \)-DCoH antiserum cross-reacts with DCoH2. A Western blot of total lysate or nuclear
extract (NE) from the indicated tissues of wild-type (+/+) or DCoH null
(−/−) mice is shown. The rightmost three lanes are lysates from COS7
cells transfected with DCoH or DCoH2 or untransfected (COS7). D, a
Western blot of 25 \( \mu \)g of total extract of derived from DCoH wild-type
(upper) or DCoH null (lower) mice from the indicated tissues probed
with \( \alpha \)-DCoH antiserum is shown.

Because DCoH enzymatic activity was severely reduced, it
was surprising that DCoH null mice did not exhibit phenotypes
similar to those observed with the loss of HNF1 activity. Al-
though DCoH stabilizes the DNA binding dimeric form of
HNF1 and augments HNF1-dependent transcription in trans-
fection assays with reporter genes (1), the necessity of cofactor
binding in the natural state was unknown. Expression of \( \alpha \)-
antitrypsin, albumin (Fig. 5), and \( \beta \)-fibrinogen and antithrom-
bin 3 (data not shown) were found to be reduced to a degree
similar to that found in HNF1\(_{\alpha}\) null mice. However, PAH RNA
levels and activity (Table 1), although reduced, were not atten-
duated to the degree observed in HNF1\(_{\alpha}\) null animals, which
have a strong hyperphenylalaninemia not caused by the loss of
BH4 recycling, but because PAH transcription is disrupted (12).
Similarly, the strong defect in IG-F-I transcription observed in
HNF1\(_{\alpha}\) null mice was not mimicked in DCoH null mice (14).
Because HNF1 activity is only partly impaired in DCoH null
mice, a complete analysis of the role of DCoH in HNF1-directed
transcription will likely require the disruption of DCoH2 ex-
pression in combination with DCoH. The complementation of
DCoH transcriptional function by DCoH2 makes it likely that
DCoH will not be a common target for mutation in MODY

**Fig. 7. Identification of DCoH2, a close homolog of DCoH.** A, sequence similarity between DCoH and DCoH2. Alignment of the
amino acid sequences of DCoH and DCoH2 in one-letter code. Identi-
tical amino acids are shaded with red, and similar amino acids as
defined by the BLAST algorithm are shaded in green. Blue indicates
strongly divergent amino acid sequence. The proteins are identical in
68% of their amino acids with 85% similarity. B, structural similarity
between DCoH and DCoH2. The crystal structure of a dimer of DCoH is
displayed using Insight II with amino acid residues that are identical or
similar between DCoH and DCoH2 colored red and strongly dissimilar
residues as shown in A colored blue. C, rabbit \( \alpha \)-DCoH antiserum cross-reacts with DCoH2. A Western blot of total lysate or nuclear
extract (NE) from the indicated tissues of wild-type (+/+) or DCoH null
(−/−) mice is shown. The rightmost three lanes are lysates from COS7
cells transfected with DCoH or DCoH2 or untransfected (COS7). D, a
Western blot of 25 \( \mu \)g of total extract of derived from DCoH wild-type
(upper) or DCoH null (lower) mice from the indicated tissues probed
with \( \alpha \)-DCoH antiserum is shown.
families particularly those that display a dominant inheritance pattern.

Acknowledgments—We thank Drs. Linda Hansen, Weidong Wang, and David Fiorentino for useful reagents. We thank S. Milstien (National Institute of Mental Health) for performing biopterin measurements. We thank Drs. Michael Bogdan, Stephen Biggar, Isabella Graef, and Greg Barsh for helpful discussions as well as Kryn Stankunas for a critical reading of the manuscript.

REFERENCES

1. Mendel, D. B., Khvari, P. A., Conley, P. B., Graves, M. K., Hansen, L. P., Admon, A., and Crabtree, G. R. (1991) Science 254, 1762–1767
2. Courtois, G., Morgan, J. G., Campbell, L. A., Fourel, G., and Crabtree, G. R. (1987) Science 238, 688–692
3. Cereghini, S., Blumenfeld, M., and Yaniv, M. (1988) Genes Dev. 2, 555–561
4. Courtois, G., Morgan, J. G., Campbell, L. A., Fourel, G., and Crabtree, G. R. (1988) FASEB J. 2, 2485–2493
5. Mendel, D. B., Hansen, L. P., Graves, M. K., Conley, P. B., and Crabtree, G. R. (1991) Genes Dev. 5, 1042–1056
6. Kuo, C. J., Conley, P. B., Chen, L. S., Sladek, F. M., Darnell, J. E., Jr., and Crabtree, G. R. (1992) Nature 355, 457–461
7. Yamagata, K., Furuta, H., Oda, N., Kaisaki, P. J., Menzel, S., Cox, N. J., Fajas, S. S., Signorini, S., Stoffel, M., and Bell, G. I. (1996) Nature 384, 458–460
8. Yamagata, K., Oda, N., Kaisaki, P. J., Menzel, S., Furuta, H., Vaxillaire, M., Southam, L., Cox, R. D., Lathrop, G. M., Morin, J. S., Oda, Y., Yano, H., Le Beau, M. M., Yamada, S., Nishigori, H., Takeda, J., Tamas, S., Hattersley, A. T., Iwaski, N., Hansen, T., Pedersen, O., Polonsky, K. S., and Bell, G. I. (1996) Nature 384, 455–458
9. Morikawa, Y., Iwaski, N., Harai, H., Furuta, H., Hinokio, Y., Cockburn, B. N., Lindner, T., Yamagata, K., Ogata, M., Tomonaga, O., Kuroki, H., Kasahara, T., Iwamoto, Y., and Bell, G. I. (1997) Nat. Genet. 17, 384–385
10. Pontoglio, M., Barra, J., Hadchouel, M., Doyen, A., Kress, C., Bach, J. P., Babinet, C., and Yaniv, M. (1996) Cell 84, 575–583
11. Pontoglio, M., Sreenan, S., Roe, M., Pugh, W., Ostrega, D., Doyen, A., Pick, A. J., Baldwin, A., Velho, G., Freguei, P., Levitsetti, M., Bonner-Weir, S., Bell, G. I., Yaniv, M., and Polonsky, K. S. (1998) J. Clin. Invest. 101, 2357–2366
12. Lee, Y. H., Sauer, B., and Gonzalez, F. J. (1998) Mol. Cell Biol. 18, 3059–3068
13. Barbacci, E., Reber, M., Ott, M. O., Breillat, C., Huetz, F., and Cereghini, S. (1999) Development 126, 4795–4805
14. Rose, R. B., Bayle, J. H., Endrizzi, J. A., Cronk, J. D., Crabtree, G. R., and Alber, T. (2000) Nat. Struct. Biol. 7, 744–748
15. Nicosia, A., Monaci, P., Tomei, L., De Francesco, R., Nuzzo, M., Stunnenberg, H., and Cortese, R. (1999) Cell 91, 1225–1236
16. Endrizzi, J. A., Cronk, J. D., Wang, W., Crabtree, G. R., and Alber, T. (1995) Science 268, 556–559
17. Fiener, R., Sauer, U. H., Stier, G., and Suck, D. (1995) EMBO J. 14, 2034–2042
18. Kim, J. L., and Burley, S. K. (1995) Structure 3, 521–534
19. Birse, D. E., Kapp, U., Strub, K., Cusack, S., and Aberg, A. (1997) EMBO J. 16, 3757–3766
20. Rhee, K. H., Stier, G., Becker, P. B., Suck, D., and Sandaltzopoulos, R. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 11891–11894
21. Hauer, C. R., Rebrin, I., Thony, B., Neuheiser, F., Curtius, H. C., Hunziker, P., Blau, N., Ghisla, S., and Heizmann, C. W. (1993) J. Biol. Chem. 268, 4829–4831
22. Kaufman, S. (1993) Annu. Rev. Nutr. 13, 261–286
23. Davis, M. D., and Kaufman, S. (1991) FERS Lett. 285, 17–20
24. Thony, B., Neuheiser, F., Kierat, L., Blaskovics, M., Arn, P. H., Fereira, P., Rebrin, I., Ayling, J., and Blau, N. (1998) Am. J. Hum. Genet. 62, 1302–1311
25. Citron, B. A., Kaufman, S., Milstien, S., Naylor, E. W., Greene, C. L., and Davis, M. D. (1993) Am. J. Hum. Genet. 53, 768–774
26. Schallerreuter, K. U., Wood, J. M., Pitzelkov, M. R., Gutarich, M., Lemke, K. R., Rodl, W., Swanson, N. N., Hitzemann, K., and Ziegler, I. (1994) Science 263, 1444–1446
27. Johnen, G., Kowlessur, D., Citron, B. A., and Kaufman, S. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 12384–12388
28. Gorski, K., Carneiro, M., and Schibler, U. (1996) Cell 77, 767–776
29. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
30. Feinberg, A., and Vogelstein, B. (1983) Anal. Biochem. 132, 6–13
31. Dukes, I. D., Sreenan, S., Roe, M. W., Levitsetti, M., Zhou, Y. P., Ostrega, D., Bell, G. I., Pontoglio, M., Yaniv, M., Philipson, L., and Polonsky, K. S. (1998) J. Biol. Chem. 273, 24457–24464
32. Pogge von Strandmann, E., and Ryffel, G. U. (1995) Development 121, 1217–1226
33. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
34. Marra, M., Hillier, L., Kuroda, T., Allen, M., Barstead, B., Beck, C., Blistain, A., Bonaldo, M., Bowers, Y., Bowles, L., Cardenas, M., Chamberlain, A., Chappell, J., Clifton, S., Favello, A., Geisel, S., Gibbons, M., Harvey, N., Hill, F., Jackson, Y., Kohn, S., Lennon, G., Mardis, E., Martin, J., and Waterston, R. (1999) Nat. Genet. 21, 191–194
35. Shedlovsky, A., McDonald, J. D., Symula, D., and Dove, W. F. (1993) Genetics 134, 1205–1210
36. Bogdan, S., Senkel, S., Esser, F., Ryffel, G. U., and Pogge v. Strandmann, E. (2001) Mech. Dev. 103, 61–69
37. Strandmann, E. P., Senkel, S., and Ryffel, G. U. (1998) Int. J. Dev. Biol. 42, 53–59
38. Resibois, A., Cuvelier, L., Svelsova, M., Heizmann, C. W., and Thony, B. (1999) Histochem. Cell Biol. 111, 381–389
39. Depeae, V., Cuvelier, L., Thony, B., and Resibois, A. (2000) Brain Res. Mol. Brain Res. 75, 76–88
40. Lei, X. D., and Kaufman, S. (1999) DNA Cell Biol. 18, 243–252
41. Pogge v. Strandmann, E., Senkel, S., and Ryffel, G. U. (2000) Mech. Dev. 91, 53–60

Knockout of DCoH in Mice