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Genetics

Marked increases in hepatic NAD(P)H:oxidoreductase gene transcription and mRNA levels correlated with a mouse chromosome 7 deletion

(DT diaphorase/albino locus/cytochrome P-450/2,3,7,8-tetrachlorodibenzo-*p*-dioxin/oxidative stress)

DANIEL D. PETERSEN*, FRANK J. GONZALEZ†, VESNA RAPIC*, CHRISTINE A. KOZAK‡, JONG-YOUN LEE*, JOHN E. JONES*, AND DANIEL W. NEBERT*

*Laboratory of Developmental Pharmacology, National Institute of Child Health and Human Development, †Laboratory of Molecular Carcinogenesis, National Cancer Institute, and ‡Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

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ABSTRACT **The NAD(P)H:menadione oxidoreductase gene (*Nmo-1*) codes for a quinone reductase (also called DT diaphorase; EC 1.6.99.2) believed to play a central role in protection against oxidative stress. We have studied mice with a radiation-induced chromosomal deletion involving the albino locus (*c*) on chromosome 7 and found that Nmo-1 mRNA levels and the rate of *Nmo-1* gene transcription are markedly increased (>100-fold and >12-fold, respectively) in the untreated c^{14CoS}/c^{14CoS} deletion homozygote, compared with the untreated c^{ch}/c^{ch} wild-type and the c^{ch}/c^{14CoS} heterozygote. These data suggest that a gene located on chromosome 7 encodes a trans-acting regulatory factor that might be a negative effector of the *Nmo-1* gene, which we show here is located on chromosome 8 approximately 1.4 centimorgans (about 1000 kilobase pairs) from the *Es-2* gene. Conversely, there are no detectable basal levels of cytochrome P_1450 (*Cyp1a1* gene) or cytochrome P_3450 (*Cyp1a2* gene) mRNA, indicating that the regulation of basal expression of the *Cyp1a1* and *Cyp1a2* genes is distinct from that of the *Nmo-1* gene. Moreover, the *Cyp1a1* and *Cyp1a2* genes and the *Nmo-1* gene are induced by tetrachlorodibenzo-*p*-dioxin in the c^{ch}/c^{ch}, c^{ch}/c^{14CoS}, and c^{14CoS}/c^{14CoS} mice. The mechanism of tetrachlorodibenzo-*p*-dioxin inducibility of the *Cyp1a1*, *Cyp1a2*, and *Nmo-1* genes is, therefore, independent of the mechanism of *Nmo-1* gene activation in untreated c^{14CoS}/c^{14CoS} mice.**

The environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is not only extremely toxic and teratogenic (1) but also a strong tumor promoter (2). TCDD exposure coordinately induces a group of genes called the *a*romatic *h*ydrocarbon-responsive [*Ah*] battery (3). This gene battery includes *Cyp1a1* (cytochrome P_1450), *Cyp1a2* (cytochrome P_3450), and *Nmo-1* [NAD(P):H-menadione oxidoreductase, EC 1.6.99.2]. The Cyp1a1 and Cyp1a2 enzymes are involved in the "phase I" oxidative metabolism of foodstuff and numerous other environmental chemicals, which paradoxically results in reactive intermediates that are often more toxic or mutagenic than their precursors (3, 4). The Nmo-1 enzyme catalyzes the "phase II" metabolism (two-electron reduction) of quinones that competes with the formation of toxic oxygenated metabolites that are generated by the Cyp1a1 and Cyp1a2 enzymes (5). Any mechanism that would differentially induce phase II enzymes with respect to phase I enzymes—which is what we show in this study—might have important consequences with regard to relative risk of toxicity, mutagenesis, and carcinogenesis.

Several groups have examined inbred mouse lines with overlapping radiation-induced chromosomal deletions involving the albino locus (*c*) on chromosome 7 and have found indirect evidence for a regulatory gene(s) located within the missing region of deletion homozygote mice (6–13). These regulatory loci appear to encode trans-acting factors that modulate the basal and inducible expression of genes that are located on other chromosomes. In most cases these genes are down-regulated 2- to 4-fold in the deletion homozygote (c^{14Cos}/c^{14CoS}), whereas the wild-type (c^{ch}/c^{ch}) and the deletion heterozygote (c^{ch}/c^{14CoS}), are unaffected (8–13). In the present study we provide evidence for a trans-acting gene that, when both copies are absent in the untreated *14CoS/14CoS* mouse, leads to marked increases in *Nmo-1* gene activation, while the *Cyp1a1* and *Cyp1a2* genes remain unaffected. This regulation of basal Nmo-1 mRNA in untreated *14Cos/14CoS* mice is independent of *Nmo-1* gene induction by TCDD. Interestingly, the Nmo-1 mRNA concentration in the untreated *14CoS/14CoS* newborn is at least 20 times greater than that induced by TCDD in the *ch/ch* or *ch/14CoS* newborn.

MATERIALS AND METHODS

Animals. Two female and one male *ch/14Cos* mice and *ch/Bi4* mice were given to us by Liane B. Russell (Oak Ridge, TN), and breeding was carried out in the mouse colony of this laboratory. Fetal livers from *ch/ch*, *ch/3H*, and *3H/3H* mice were provided by Salome Gluecksohn-Waelsch (Albert Einstein College of Medicine, Bronx, NY). For the enzyme activities and mRNA levels in this study, the background strains of these deletion alleles (C3H and 101, respectively) were found not to differ from the *ch/ch* parent. Mice (*Mus musculus musculus*) were provided by Michael Potter from his Hazelton Laboratories (Rockville, MD) colony, and NFS/N mice were obtained from the Veterinary Resources Branch, National Institutes of Health (Bethesda, MD).

TCDD treatment (2 μg/kg) consisted of a single intraperitoneal injection given to the pregnant mice 24–48 hr before killing; the inducer is known to cross the placenta in 1–2 hr and induce these enzymes in fetal liver (14). Control mice

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Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; B6, C57BL/6 inbred mouse strain; D2, DBA/2 inbred mouse strain; *Nmo-1*, TCDD-inducible mouse gene for NAD(P)H:menadione oxidoreductase; *Cyp1a1*, mouse gene for cytochrome P_1450; *Cyp1a2*, mouse gene for cytochrome P_3450; *Ah*, *a*romatic *h*ydrocarbon responsiveness; *Pepck*, mouse gene for phospho*enol*pyruvate carboxykinase; *Ugt-1*, TCDD-inducible mouse gene for UDPglucuronosyltransferase with *p*-nitrophenol as substrate; *Gt-1*, TCDD-inducible mouse gene for glutathione transferase with 1-chloro-2,4-dinitrobenzene as substrate.

received the vehicle p-dioxane (25 μl/kg) alone. The control and TCDD-treated mothers were used for the adult mouse liver samples. At least three experiments were carried out on separate occasions (mother and litters) and are regarded as different samples ($n = 3$).

Enzymes. Enzyme assays corresponding to Nmo-1 (15) and Cyp1a1 (16) have been detailed. The activities are expressed as nmol of cytochrome c reduced per min per mg of cytosolic protein and pmol of hydroxylated benzo[a]pyrene per min per mg of microsomal protein, respectively.

Northern Blot Analysis. RNA was isolated from individual livers by a modification of the procedure (17). The tissue was solubilized in 8 M guanidinium thiocyanate, and RNA was sedimented through a cushion of cesium trifluoroacetate (Pharmacia). The pellet was resuspended in 0.3 M sodium acetate (pH 6.0) and the RNA was precipitated with ethanol. The RNA was resolved on gels containing 1% agarose and 2.2 M formaldehyde (18) and blotted to Nytran membranes (Schleicher & Schuell). Hybridization and washing of filters was performed as described (19). The filters were exposed to x-ray film with the aid of a DuPont Lightning Plus intensifying screens.

The Nmo-1 probe was the 1.14-kilobase (kb) *Pvu* II–*Hin*dIII fragment removed from the rat 1501-base-pair full-length Nmo-1 cDNA; this probe includes all of the translated region and hybridizes with the orthologous 1.6-kb mouse Nmo-1 mRNA (15). The Cyp1a1 and Cyp1a2 mRNAs (2.9 and 2.1 kb, respectively) are both detectable with the mouse P_1450 full-length cDNA probe (20). The phospho*enol*pyruvate carboxykinase (*Pepck* gene) cDNA (21) was a gift of Daryl K. Granner (Vanderbilt University, Nashville, TN). The rat β-actin probe, used to standardize the amount of newborn hepatic RNA applied per lane, was given to us by Bruce Paterson (National Cancer Institute, Bethesda).

Transcription Run-On Assays. The rate of transcription of the *Nmo-1, Cyp1a1, Pepck,* and β-actin genes was assayed *in vitro* by a modification of the published nuclear run-on procedure (22), as performed in this laboratory (23). Briefly, hepatic cell nuclei were prepared from newborn mice and incubated with [^{32}P]UTP. Labeled RNA transcripts were purified and hybridized to the cDNA probes that had been immobilized on nitrocellulose filter disks. RNAs transcribed *in vitro* and specifically retained by the filters were analyzed by liquid scintillation spectrometry. The data were measured as specific radioactivity hybridized in parts per million (ppm) and expressed as the fold induction (*14CoS/14CoS*:*ch/ch*) after subtraction of α-amanitin-insensitive transcripts.

Chromosomal and Subchromosomal Localization. Preparation of the mouse × Chinese hamster somatic cell hybrids has been described (24–26). Genomic DNA (10 μg) was digested with *Bam*HI, separated on a 0.7% agarose gel, and transferred to nylon [Biotrace] filters. DNA samples were hybridized with the 1.14-kb Nmo-1 cDNA probe described above. The probe was labeled with [α-^{32}P]dCTP by the random-oligonucleotide-priming procedure (27) to a specific activity of 10^9 dpm/μg of DNA. Routine conditions for the hybridization and washing of filters were used (28). For subchromosomal localization, the NFS/N × *M. musculus* crosses and backcrosses were performed at the National Institutes of Health (Bethesda, MD), and DNA was isolated from 67 mice.

RESULTS

Nmo-1 and Cyp1a1 Enzyme Activities. Our initial observation was that the basal Nmo-1 enzyme activity was >25 times greater in the untreated *14CoS/14CoS* mouse than the *ch/ch* mouse or the *ch/14CoS* mouse (Table 1). These results suggest that the deletion homozygote mouse might have lost both copies of a regulatory gene encoding a factor that is a negative effector of the Nmo-1 activity. It is, of course, conceivable that the deletion homozygote may have lost both copies of a gene that positively controls the degradation of the Nmo-1 mRNA or enzyme. These hypotheses are tested below.

Table 1. Nmo-1 and Cyp1a1 enzyme activities

Mice	Nmo-1 activity		Cyp1a1 activity	
	Control	TCDD	Control	TCDD
Newborn				
B6	61 ± 21	130 ± 27	11 ± 6	2300 ± 280
D2	27 ± 6	21 ± 4	33 ± 8	24 ± 12
ch/ch	32 ± 6	94 ± 22	5 ± 3	2600 ± 550
ch/14CoS	37 ± 9	69 ± 8	7 ± 3	3000 ± 470
14CoS/14CoS	960 ± 150	3200 ± 30	4 ± 2	2100 ± 810
Adult B6	93 ± 17	330 ± 8	98 ± 18	2200 ± 400

Each sample represents the cytosolic (Nmo-1 activity) or microsomal (Cyp1a1 activity) fractions from three or four newborn livers combined or one adult liver. Three independent experiments in duplicate were performed (n=3); values are expressed as mean ± SEM. Units for Nmo-1 activity are nmol of cytochrome c reduced per min per mg of cytosolic protein. Units for Cyp1a1 activity are pmol of hydroxylated benzo[a]pyrene per min per mg of microsomal protein.

Table 1 also shows that the Nmo-1 and Cyp1a1 enzyme activities in the C57BL/6 (B6) newborn mouse are induced transplacentally by TCDD >3- and 200-fold, respectively; these activities are not induced in the DBA/2 (D2) newborn mouse. This B6–D2 genetic difference is consistent with data (3) indicating that the Nmo-1 and Cyp1a1 enzymes are encoded by genes in the TCDD-inducible [*Ah*] battery and that the B6 mouse has a high-affinity Ah receptor that effectively binds the inducer TCDD (and other inducers such as benzo[a]pyrene and plant flavones), whereas the D2 mouse has a low-affinity Ah receptor. TCDD inducibility and the relative fold induction of the Nmo-1 and Cyp1a1 enzyme activities is *ch/ch, ch/14CoS,* and *14CoS/14CoS* mice appear to be similar to those seen in B6 mice, indicating that these mice have a high-affinity rather than a low-affinity Ah receptor. These data further suggest that the enhancement of basal Nmo-1 activity is independent of TCDD inducibility. Moreover, there is no increase in the Cyp1a1 activity from the untreated *14CoS/14CoS* mouse, indicating that the regulation of basal expression of the Nmo-1 enzyme might be different from that of the Cyp1a1 enzyme.

Basal mRNA Levels. To delineate further the molecular mechanisms leading to the increased Nmo-1 and Cyp1a1 enzyme activities in untreated *14CoS/14CoS* mice, we measured the mRNA levels of each of these genes. Liver RNAs from TCDD-treated and control mice were probed with cDNAs specific for *Nmo-1, Cyp1a1,* and *Cyp1a2*. In untreated *14CoS/14CoS* newborns, basal Nmo-1 mRNA levels are markedly elevated, relative to *ch/ch* or *ch/14CoS* newborns (Fig. 1). Scanning densitometry of autoradiograms, standardized by the amount of newborn hepatic β-actin mRNA in each lane as a control, revealed that the Nmo-1 mRNA concentration in *14CoS/14CoS* mice is increased >100-fold, compared with the untreated wild-type or deletion heterozygote.

On the other hand, there is no detectable Cyp1a1 or Cyp1a2 mRNA in the untreated *14CoS/14CoS, ch/ch,* or *ch/14CoS* newborns (Fig. 1). These gels were exposed to films for 48 hr; exposure for 6 weeks still revealed no detectable Cyp1a1 mRNA. Trace levels of constitutively expressed Cyp1a2 mRNA were found in all newborn samples after long exposure times. Western immunoblot analysis of Cyp1a1 and Cyp1a2 proteins from B6, *ch/ch, ch/14CoS,* and *14CoS/14CoS* liver microsomes was consistent with the Cyp1a1 and Cyp1a2 mRNA results; proteins were only detected when, on Northern transfers, the mRNA was also detected (data not shown).

Gene Transcription. A better understanding of the mechanism of Nmo-1 mRNA increases in the *14Cos/14CoS* mice was afforded by transcription run-on experiments. The rate of *Nmo-1* gene transcription was found to be >12-fold greater in

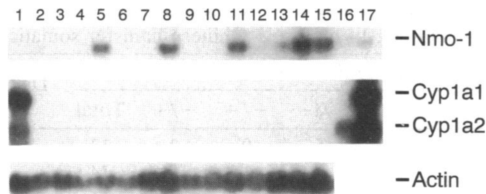

FIG. 1. Northern hybridization analysis of liver RNA from untreated *ch/ch* (lanes 7, 10, and 13), *ch/14CoS* (lanes 6, 9, and 12), and *14CoS/14CoS* (lanes 5, 8, 11, 14, and 15) newborns and from B6 and D2 mice as follows. Lanes: 1, TCDD-treated B6 newborn; 2, control B6 newborn; 3, TCDD-treated D2 newborn; 4, control D2 newborn; 16, control B6 maternal liver; 17, TCDD-treated B6 maternal liver. Each lane represents RNA from an individual mouse. Probes include Nmo-1 cDNA, full-length Cyp1a1 cDNA that detects both Cyp1a1 and Cyp1a2 mRNAs, and β-actin cDNA as control.

untreated *14CoS/14CoS* than untreated *ch/ch* mice (Fig. 2). Because the difference in Nmo-1 mRNA levels between these mice is >100-fold, perhaps both a transcriptional component and a posttranscriptional component cause levels of Nmo-1 mRNA and enzyme activity to be markedly elevated in the deletion homozygote mice, as compared with those in wild-type mice.

The transcriptional rates of the *Cyp1a1* gene and the control β-actin gene are not significantly different between untreated *14CoS/14CoS* and *ch/ch* mice, whereas the rate of *Pepck* gene transcription in untreated *14CoS/14CoS* mice is about twice the rate in *ch/ch* (Fig. 2).

Nmo-1, Cyp1a1, and Cyp1a2 Induction by TCDD. We next examined the possible relationship between the increases in Nmo-1 mRNA in untreated *14CoS/14CoS* mice and the increases in Nmo-1 mRNA brought about by TCDD induction. We found that the mRNA levels from all three of these genes were increased in TCDD-treated newborn B6 mice but not in TCDD-treated newborn D2 mice (Fig. 1). In the *ch/ch*, *ch/14CoS*, and *14Cos/14CoS* newborns, TCDD induced Nmo-1, Cyp1a1, and Cyp1a2 mRNA (Fig. 3). TCDD induction of Nmo-1 mRNA ranged between 3- and 5-fold, whereas TCDD induction of Cyp1a1 and Cyp1a2 mRNA was 30- to 100-fold. Enhanced constitutive levels of Cyp1a2 mRNA (Fig. 1, lane 16) are known to occur in adult liver (3). Cyp1a1 mRNA induction by TCDD transplacentally (Fig. 3, lanes 2 and 3) can be measured at 16 days of gestation and earlier, whereas Cyp1a2 mRNA induction by TCDD does not occur until the newborn period (29, 30). The Northern blots (Figs. 1 and 3) reinforce the enzyme activity data (Table 1) in showing that the basal *Nmo-1* gene expression in untreated *14CoS/14CoS* mice is independent of the TCDD induction

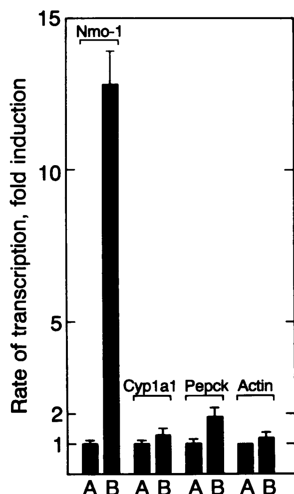

FIG. 2. Transcriptional regulation of four genes in mouse liver. The rate of transcription in *14CoS/14CoS* newborns (bars B) is compared with that in *ch/ch* newborns (bars A); results are expressed as the fold induction between the untreated radiation-deletion mutant and the untreated wild type.

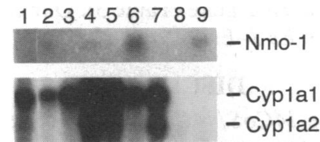

FIG. 3. Northern hybridization analysis of hepatic RNA from TCDD-treated (lanes 1–7) and control (lanes 8 and 9) B6, *ch/ch*, *ch/14CoS*, and *14CoS/14CoS* mice. Each lane represents RNA from an individual mouse. Total gestation in the mouse is 21 days. Lanes: 1, B6 newborn; 2, *14CoS/14CoS* at 16 days of gestation; 3, *ch/ch* at 16 days of gestation; 4, *ch/14CoS* newborn; 5, 7, and 8, *ch/ch* newborn; 6 and 9, *14CoS/14CoS* newborn.

process and that basal expression of the *Nmo-1* gene differs from that of the *Cyp1a1* and *Cyp1a2* genes.

Elevation of the Nmo-1 mRNA in untreated *14CoS/14CoS* mice was so striking that the 3- to 5-fold induction of Nmo-1 mRNA by TCDD was overshadowed. In the 16-day-old liver (Fig. 3, lanes 2 and 3), the Nmo-1 mRNA levels were elevated in the *14Cos/14CoS*, compared with the *ch/ch* mouse. This observation demonstrates that the mechanism of Nmo-1 mRNA elevation in *14CoS/14CoS* mice is not an event that occurs only in the neonatal period but can easily be detected 5–6 days before birth.

Screening of Radiation-Deletion Lines. To define more precisely the chromosomal location of the putative trans-acting gene, we screened three mutant mouse lines having deletions that include the *c* locus (Fig. 4). Nmo-1 mRNA was found to be markedly elevated in the untreated *3H/3H* and the *14CoS/14CoS* but not the *Bi4/Bi4* deletion homozygote. We, therefore, postulate that a gene encoding a putative trans-acting regulatory factor that negatively controls the *Nmo-1* gene is located on chromosome 7 within an ≈1.1-centimorgan (cM; 1 cM = 1% recombination or 1000 kilobase pairs) region (Fig. 4).

Chromosomal and Subchromosomal Localization of the *Nmo-1* Locus. Since the putative trans-acting regulatory gene is presumed to be on chromosome 7, we wished to determine the chromosomal location of the *Nmo-1* gene. By using genomic DNA from 14 mouse × hamster hybrids and the parent lines for Southern hybridization analysis (Fig. 5*A* and Table 2), we localized the *Nmo-1* gene to mouse chromosome 8. There were no hybrid cell lines discordant for the cosegregation of chromosome 8 and the Nmo-1 cDNA, whereas all other chromosomes showed ≥16.7% discordancies.

DNA from 67 backcross mice was digested with various restriction endonucleases and subjected to Southern blot hybridization analysis with the Nmo-1 cDNA probe. No restriction length polymorphisms were found between NFS and *M. musculus* mice for the enzymes *Bam*HI, *Hin*dIII, *Eco*RI, *Pvu* II, *Xba* I, *Pst* I, *Sac* I, or *Bgl* II. We used an *Apa* I polymorphism (Fig. 5*B*) to map the *Nmo-1* gene to a location

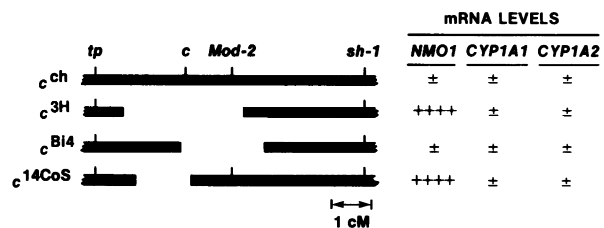

FIG. 4. Diagram of a small region around the albino (*c*) locus on chromosome 7, the approximate size of the deletion in the three mouse lines studied in this report (taken from data reviewed in refs. 6, 9, and 10), and the Nmo-1, Cyp1a1, and Cyp1a2 mRNA concentrations found in the untreated wild-type homozygote (*ch/ch*) and the three deletion homozygotes examined. *tp*, gene for taupe coat color; *Mod-2* locus, mitochondrial malic enzyme; *sh-1*, gene for shaker-1. ±, Below level of detection; ++++, >100-fold increases.

≈1.4 cM from the *Es-2* gene (Table 3). Our data indicate that the gene order is *Es-1–Es-2–Nmo-1*.

DISCUSSION

Studies with the *14CoS/14CoS* mouse have suggested that the deleted chromosomal region (≈1.2 cM) may contain gene(s) encoding one or more positive trans-acting factor(s). It has been estimated that ≈30 genes would be present in a region of 1.0 cM of genomic DNA (31); thus, we would expect that the *14CoS/14CoS* deletion might comprise <36 genes. The expression of glucose 6-phosphate (10) and tyrosine aminotransferase, *Pepck* induction by glucocorticoids (11, 12), and metallothionein expression (13) are 2- to 4-fold decreased in the *14CoS/14CoS* mouse, compared with the *ch/ch* and the *ch/14CoS* mouse, suggesting a gene on chromosome 7 that might encode one or more trans-acting positive regulatory factor(s). The expression of UDPglucuronosyltransferase activity with *p*-nitrophenol as substrate (*Ugt-1*) and glutathione transferase activity with 1-chloro-2,4-dinitrobenzene as substrate (*Gt-1*) is about twice higher in *14CoS/14CoS* than in *ch/ch* or *ch/14CoS* newborns (6, 7), suggesting a gene on chromosome 7 that codes for a trans-acting negative regulatory factor. Regulatory proteins can have a positive effect on transcription under one and a negative effect under another set of circumstances (cf. ref. 32 and references therein). It is therefore conceivable, although quite unlikely, that the elevated expression of Nmo-1 mRNA and of Ugt-1 and Gt-1 enzyme activities (6, 7), as well as the decreased expression of the four genes described above (10–13) represents the action of the same gene that is missing in the *14CoS/14CoS*-deleted chromosomal region.

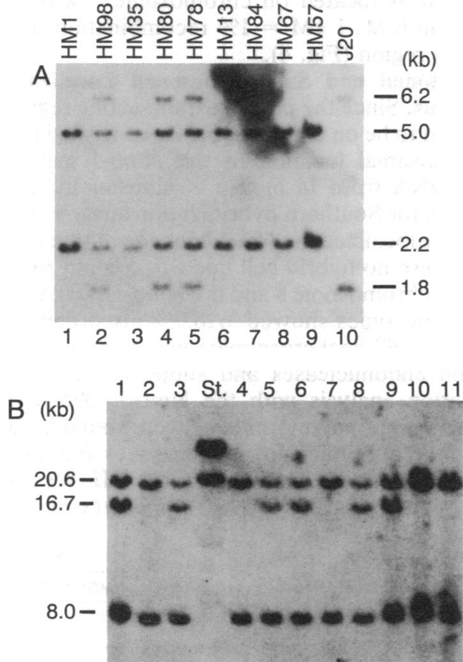

FIG. 5. Southern hybridization analysis. (*A*) DNA from the hamster parent line HM1 (lane 1), the mouse parent line J20 (lane 10), and eight representative hybrid cell lines (lanes 2–9) as indicated. Genomic DNA was cleaved with *Bam*HI, and rat NMO1 cDNA probe was used. The corresponding mouse *Nmo-1* gene can be seen in lanes 2, 4, 5, and 10. (*B*) *Apa* I-cleaved DNA from 11 representative mice (lanes 1–11) of the 67 progeny from the NFS/N × *M. musculus* backcross. Lane St. contains standards from λ DNA. The mixed probe included the *Nmo-1* 1.14-kb fragment plus λ DNA. The 20.6- and 16.7-kb bands represent the NFS/N and *M. musculus* alleles, respectively; the 8.0-kb band is common to both parents. Lanes 1, 3, 5, 6, 8, and 9 have the heterozygote *Apa* I polymorphism pattern.

Table 2. Correlation between mouse chromosome 8 and the *Nmo-1* gene among 14 mouse × Chinese hamster somatic hybrids

Mouse chromosome	Hybrids with the correlation, no.					Discordance, %
	+/+	−/−	+/−	−/+	Total	
1	5	5	0	2	12	16.7
2	6	2	1	5	14	42.9
3	3	5	2	1	11	27.3
4	4	6	3	1	14	28.6
5	2	7	3	0	12	25.0
6	6	3	1	3	13	30.8
7	6	1	1	6	14	50.0
8	6	5	0	0	11	0
9	4	4	2	2	12	33.3
10	1	6	4	0	11	36.4
11	0	5	3	0	8	37.5
12	3	1	1	4	9	55.6
13	5	2	0	5	12	41.7
14	1	5	6	2	14	57.1
15	4	0	0	5	9	55.6
16	2	6	2	1	11	27.3
17	5	2	0	4	11	36.4
18	5	5	1	2	13	23.1
19	6	3	1	2	12	25.0
X	6	5	1	2	14	21.4

Eight of the 14 hybrids have been karyotyped; 6 were typed for the presence or absence of specific marker loci (24–26). The number of hybrid clones with sequences homologous to the mouse *Nmo-1* gene vs. chromosome retention (the correlation) was obtained. +/+, Contains sequences that hybridize to Nmo-1 cDNA and the indicated mouse chromosome; −/−, lacks sequences that hybridize to Nmo-1 cDNA and the indicated mouse chromosome; +/−, contains sequences that hybridize to Nmo-1 cDNA but lack the indicated mouse chromosome; and −/+, lacks sequences that hybridize to Nmo-1 cDNA but contains the indicated mouse chromosome.

Basal Nmo-1 and Cyp1a1 enzyme activities in liver, especially adult liver, are known to represent two or more enzymes encoded by different NAD(P)H:menadione oxidoreductase genes and P450 genes, respectively (3, 15). Therefore, fold inducibility of these enzyme activities (Table 2) cannot be directly compared with fold inducibility of the mRNAs that hybridize to distinct cDNA probes. The same can probably be said for the Ugt-1 (6) and Gt-1 (7) enzyme activities; if the specific mRNA instead of enzyme activity can be measured, it is likely that the expression of the *Ugt-1* and *Gt-1* genes will be >2-fold increased in *14CoS/14CoS* newborns as compared with *ch/ch* and *ch/14CoS* newborns.

It must be emphasized, however, that the small 2-fold changes in Ugt-1 and Gt-1 enzyme activities measured in these early experiments might reflect secondary effects based on the lethal nature of the mutations in these radiation-deletion mice. For instance, cellular stress may cause an indirect increase in some enzymes. In contrast, in the present

Table 3. Subchromosomal localization of the mouse *Nmo-1* gene on chromosome 8

	Gene			Number
	Es-1	*Es-2*	*Nmo-1*	
Parental	+	+	+	46
	−	−	−	18
Recombinant	+	−	−	1
	−	+	+	1
	−	−	+	1

+, Inheritance of the *Nmo-1* gene *Apa* I polymorphism and *Es-1* and *Es-2* isozyme variants identical to that in the NFS/N parent; −, inheritance of corresponding sequences of the *M. musculus* parent. The distance between markers is as follows: *Es-1* to *Es-2*, 2/67 → 2.9 ± 2.0 cM; *Nmo-1* to *Es-1*, 3/67 → 4.4 ± 25 cM; *Nmo-1* to *Es-2*, 1/67 → 1.4 ± 1.4 cM.

study we have measured dramatic increases in transcription, mRNA levels, and enzyme activity (>12-fold, >100-fold, and >25-fold, respectively) of the *Nmo-1* gene that most likely represent the loss of a trans-acting negative regulatory protein in *14CoS/14CoS* mice. To our knowledge, increases of this magnitude have not been seen before with any other gene in these radiation-deletion mice.

It is noteworthy that the maximal levels of TCDD-induced Nmo-1 mRNA are <5% of the levels detected in the untreated *14CoS/14CoS* mouse (Fig. 3). The >12-fold increase in *Nmo-1* gene transcription might reflect a strong *Nmo-1* promoter that, when released from repression in the untreated *14CoS/14CoS* mouse, produces striking increases in the transcriptional rate. As with many other genes, a large discrepancy has been found between the difference in mRNA levels (>100-fold) and transcriptional rate (>12-fold), suggesting a posttranscriptional, in addition to a transcriptional, mechanism of *Nmo-1* gene activation. The potent inducer TCDD was given at a dose known (15) to induce the *Nmo-1* gene maximally, yet Nmo-1 mRNA is stimulated to levels that are ≈5% of that seen in the untreated *14CoS/14CoS* mouse. The presence of this putative trans-acting negative regulatory protein may, therefore, modulate the level of *Nmo-1* inducibility by TCDD.

The human *NMO1* (*DIA4*) gene is known to reside on chromosome 16 (33). Our localization of the mouse *Nmo-1* gene to chromosome 8 near the *Es-2* locus (Tables 2 and 3) is another manifestation of the homology shown to exist between mouse chromosome 8 and human chromosome 16 (34–36). Localization of *Cyp1a1* and *Cyp1a2* genes near the *Mpi-1* locus on mouse chromosome 9 has been reported (37). The mouse *Ah* locus encoding the Ah receptor, a positive effector of the *Nmo-1*, *Cyp1a1*, and *Cyp1a2* genes, has been localized to chromosome 12 (38, 39). These data are thus consistent with our finding (Fig. 3) that the Ah receptor-mediated TCDD inducibility of the *Nmo-1* gene is independent of the marked activation of the *Nmo-1* gene caused by the homozygous deletion of a portion of chromosome 7. Our findings suggest that at least two independent mechanisms operate in *Nmo-1* gene activation.

Nmo-1 activity is known to prevent the formation of highly mutagenic quinone metabolites (40), to detoxify benzo[a]pyrene-3,6-quinone by glucuronide formation (41), to lower chromium(VI) mutagenicity by reduction (42), and to decrease the covalent binding of oxygenated metabolites to microsomal proteins (43). Regulation of the *Nmo-1* gene by the locus on mouse chromosome 7 encoding the trans-acting negative regulatory factor may, therefore, be important in the cell's capacity to survive stress from oxidative metabolites and might potentially act as an important antioncogene in conferring protection from environmental mutagens. The *Nmo-1* gene and the two genes encoding hemoprotein monooxygenases, *Cyp1a1* and *Cyp1a2*, are members of the TCDD-inducible [*Ah*] gene battery. In yeast, a binding site for the HAP1 protein, which regulates the *CYC1* and *CYC7* mitochondrial cytochrome genes, has been found in the upstream activating sequences region of the catalase T gene, suggesting a possible coordinated regulation of hemoproteins involved in the detoxification of oxygen metabolites (44). Further characterization of the missing gene(s) near the *c* locus on mouse chromosome 7 in *14CoS/14CoS* mice should shed additional light on our fundamental understanding of the cell's coordinated response to oxidative stress.

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