www-shgc.stanford.edu/RH/rhserver_form2.html, and the computed results were returned to us.

Results: This typing of the radiation hybrid panel showed that the human MN/CA9 locus is located on chromosome 9, 3.33 cR_{10000} from SHGC-10717 (lod score >15.0) (Fig. 1). From the location data of neighboring marker loci, it is inferred that the human MN/CA9 locus is located between 57 and 59 cM on the genetic map from the top and on 9p12–p13 cytogenetically.

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Localization of the Type 3 Iodothyronine Deiodinase (*DIO3*) Gene to Human Chromosome 14q32 and Mouse Chromosome 12F1

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Functional gene description: The iodothyronine deiodinases constitute a family of enzymes that metabolize thyroid hormones by the removal of an iodine from either the phenolic or the tyrosyl ring (5). Three deiodinase isoforms have been identified, all of which contain the uncommon amino acid selenocysteine at the catalytic site. The type 3 iodothyronine deiodinase (D3), encoded in human by the *DIO3* gene, catalyzes exclusively tyrosyl ring deiodination, which results in the conversion of thyroxine (T_4) and 3,5,3′-triiodothyronine (T_3) to the metabolically inactive products 3,3′,5′-triiodothyronine and 3,3′-diiodothyronine, respectively (5). During development, D3 is highly expressed in the placenta and several fetal tissues, and it appears to function to protect the mammalian embryo from premature exposure to adult levels of active thyroid hormones (5). In the adult, D3 expression is limited to the skin and central nervous system. To begin to understand the genetic factors responsible for this pattern of expression, we have identified human and mouse D3 gene fragments and have mapped the chromosomal location of this gene in these species.

Description of clones: The rat NS27-1 D3 cDNA, described previously (3), was used to probe a murine (129SVJ strain) genomic library constructed in the Lambda Dash II vector (Stratagene). A 12-kb fragment was identified, characterized by restriction mapping, and partially sequenced. The sequence data were used to design oligonucleotide primers for the isolation of a mouse P1 clone (Genome Systems) by a PCR-based screening system. The mouse primers used in the reaction were derived from the exonic sequence of the mouse gene: 5′ primer, 5′-CGCCATCCTGACCACCCTGA-3′; 3′ primer, 5′-AAATTGAGCACCAACGGGCG-3′. A human P1 clone was obtained in the same manner using primers (5′ primer, 5′-CGCCCAGACCGCCTCGT-3′; 3′ primer, 5′-AAATTGAGAACCAGCGGGCG-3′) derived from the published sequence of the human D3 cDNA (*vide infra*). The mouse and human P1 clones were used as probes for fluorescence *in situ* hybridization (FISH).

Methods used to validate gene identity: Restriction fragments of the mouse and human P1 clones were subcloned, sequenced, and compared to published sequences for the rat and human D3 cDNAs (GenBank Accession Nos. U24282 and S79854, respectively). In addition, the mouse P1 sequence was compared to that of the murine 129SVJ genomic clone originally isolated.

Methods of mapping: Human and mouse P1 clones specifying the D3 gene were used to determine the chromosomal localization of the gene in these two species by FISH. Target chromosomes for FISH were prepared by standard techniques from normal human lymphocytes and mouse fibroblast cultures. The P1 clones were biotin labeled by nick-translation (Bionick, Gibco BRL). The hybridization solution contained 0.2 μg labeled probe, 10 μg Cot-1 DNA (Gibco BRL), and 30 μg herring sperm DNA (Gibco BRL) in 15 μl of Hybrisol VII (Oncor) per slide. For the mouse FISH, chromosome 12 was identified with a digoxigenin-labeled paint probe (Oncor, Catalog No. P6112.dg), the specificity of which was documented by the supplier using cohybridization with a mouse chro-

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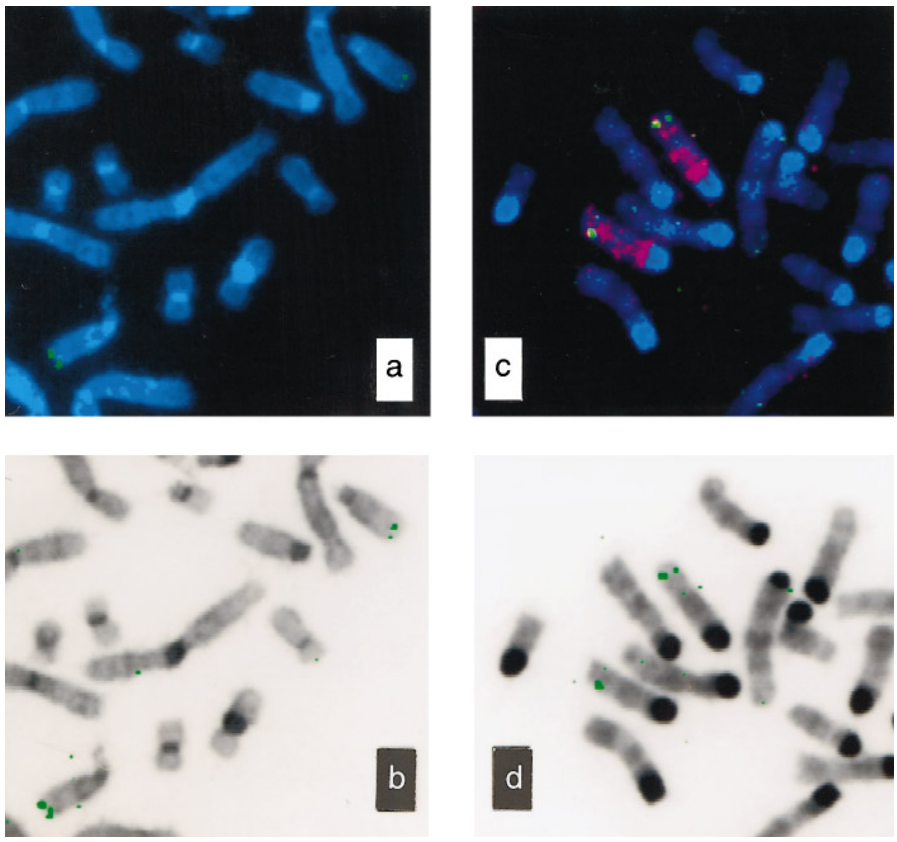

FIG. 1. Chromosomal localization of human *DIO3* and mouse *Dio3*. (**a**) A partial metaphase showing hybridization of the *DIO3* probe to human chromosome 14. (**b**) The partial metaphase in **a** with hybridization signal overlaid on pseudo-G-banded chromosomes, demonstrating localization of *DIO3* to band 14q32. (**c**) A partial metaphase showing dual-color hybridization of the mouse *Dio3* probe (yellow) and chromosome 12 paint (red) probe. (**d**) The partial metaphase in **c** with hybridization of the *Dio3* probe overlaid on pseudo-G-banded chromosomes, demonstrating localization of *Dio3* to band 12F1.

mosome 12-specific telomere probe. Ten microliters of this latter probe was added to the hybridization solution. The probe cocktail was heat denatured at 70°C for 5 min and allowed to preanneal at 37°C for 2 h. Chromosome preparations on slides were conditioned prior to hybridization by a 30-min 37°C bath in 2× SSC followed immediately by dehydration in 70, 80, and 95% EtOH (2 min each) at room temperature and air-dried. The slides were then denatured in 70% formamide/2× SSC at 70°C for 5 min followed by serial dehydration at room temperature. Hybridization was performed for 18 h in a moist, 37°C chamber. Slides were washed in 2× SSC at 72°C for 5 min. Slides were further washed three times at room temperature in phosphate-buffered detergent prior to signal detection. For the mouse FISH, chromosome 12 paint was detected with rhodamine and anti-digoxigenin following a single round of amplification according to the supplier's instructions. The biotin-labeled P1 probes were detected with avidin-FITC following a single round of amplification. FISH signals were captured using a monochromatic CCD camera mounted on a Zeiss epifluorescence microscope with a LUDL filter wheel and a fixed, multibandpass beam splitter using MacProbe software (PSI, Houston, TX).

Results: For the human mapping, analysis of 25 metaphases showed that 13 cells had four signals, 11 had three signals, and 1 had two signals at band 14q32 (Fig. 1a). No background signals (sites with more than two signals) were observed. The localization of the *DIO3* signal to 14q32 was verified by digitally reversing the DAPI staining pattern to achieve pseudo-G-banding (Fig. 1b).

Chromosomal localization of mouse *Dio3* was achieved by dual-color FISH (Fig. 1c). Human–mouse chromosome homology relationships predicted that mouse *Dio3* will map to chromosome 12. To facilitate chromosome identification, digoxigenin-labeled mouse chromosome 12 paint probe was cohybridized with the biotin-labeled mouse *Dio3* probe. Analysis of 25 metaphases showed that 15 cells had four signals, 7 had three signals, and 3 had two signals at band 12F1 (Fig. 1c). No background signals (sites with more than two signals) were observed. The localization of the *Dio3* signal to 12F1 was verified by digitally reversing the DAPI staining pattern to achieve pseudo-G-banding (Fig. 1d).

Homologies: The localization of human *DIO3* to 14q32 and mouse *Dio3* to 12F1 is in keeping with the established homologies between these two chromosomal regions from comparative gene mapping studies. The products of these mouse and human genes show homology to the type 1 and type 2 iodothyronine deiodinases expressed in these same species; amino acid identity between any two given isoforms within the same species is 29–39%. At the nucleotide level, the coding regions of *DIO2* and *DIO3* show less than 40% identity (5).

Dio1 has previously been mapped to mouse chromosome 4 (1) and *DIO1* to human chromosome 1p32–p33 (4). *DIO2* has recently been mapped to human chromo-

some 14q24.3 by screening of radiation hybrid panels (2). The assigned location of *DIO2* thus represents an estimate of the cytogenetic location, and it remains uncertain as to whether *DIO2* and *DIO3* are clustered in the distal 14q region. Of note, Southern blotting of the mouse and human *DIO3* P1 clones used in our study with *DIO2* cDNA probes did not identify any reactive bands (data not shown).

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