

# Molecular structure and chromosomal mapping of the human homolog of the agouti gene

(physical mapping/maturity onset diabetes of the young)

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**ABSTRACT** The agouti (*a*) locus in mouse chromosome 2 normally regulates coat color pigmentation. The mouse agouti gene was recently cloned and shown to encode a distinctive 131-amino acid protein with a consensus signal peptide. Here we describe the cloning of the human homolog of the mouse agouti gene using an interspecies DNA-hybridization approach. Sequence analysis revealed that the coding region of the human agouti gene is 85% identical to the mouse gene and has the potential to encode a protein of 132 amino acids with a consensus signal peptide. Chromosomal assignment using somatic-cell-hybrid mapping panels and fluorescence *in situ* hybridization demonstrated that the human agouti gene maps to chromosome band 20q11.2. This result revealed that the human agouti gene is closely linked to several traits, including a locus called *MODY* (for maturity onset diabetes of the young) and another region that is associated with the development of myeloid leukemia. Initial expression studies with RNA from several adult human tissues showed that the human agouti gene is expressed in adipose tissue and testis.

The agouti (*a*) locus in mouse chromosome 2 normally regulates the differential production of eumelanin (black or brown) and pheomelanin (yellow) pigment granules by the melanocytes within the hair follicle. Unlike the homogeneous black or yellow coat coloration associated with certain mouse mutants, agouti pigmentation arises from individual hair shafts containing a subapical yellow band on an otherwise black or brown background (1) and is conserved in many mammalian species (2). We and others recently cloned the agouti gene and found that it is normally expressed in neonatal skin (3, 4). It encodes a small protein containing a consensus signal peptide, indicating that the agouti protein is probably secreted.

Several dominant agouti alleles (e.g., *A<sup>y</sup>*, *A<sup>vy</sup>*, *A<sup>sv</sup>*, *A<sup>bv</sup>*) cause a predominantly yellow pelage (1), as well as a number of additional effects that include a non-insulin-dependent diabetic-like condition, pronounced obesity, and a marked increase in the incidence of spontaneous and induced tumors (1, 8–11). Assessment of the physiology of these mutants revealed that their diabetic/obesity condition has a notable similarity to non-insulin-dependent diabetes in humans (11, 12). We previously demonstrated that several of these mutant alleles exhibit deregulated agouti gene expression caused by structural alterations within the noncoding regions of the gene (3, 13). Based on this finding, we hypothesized that ectopic overexpression of the agouti protein in certain tissues

of the animal was responsible for the dominant pleiotropic effects associated with these mutants.

Although the agouti banded pigmentation pattern is conserved in many mammalian species (2), there is no reported evidence that humans ever develop agouti pigmented hair. For this reason, it was initially unclear whether humans have an agouti gene. On the other hand, the similarities between the phenotype in dominant agouti mutants and non-insulin-dependent diabetes in humans raised the question of whether a human agouti gene, if it exists, could be involved in certain forms of diabetes and/or obesity in humans. Additionally, the association between the ectopic expression of agouti and the development of tumors in mice led to the question of whether the occurrence of any human tumors might be associated with the deregulated expression of a human agouti gene. For these reasons, we set out to determine whether humans contain an agouti gene and to clone and characterize the structure of such a gene, if it exists.

Here we report that the human genome does contain an agouti gene, that it is highly homologous to the mouse gene, and that it maps to a region of chromosome 20 that exhibits synteny conservation with the corresponding region of mouse chromosome 2 (14, 15). This portion of human chromosome 20 is closely linked to several traits, including a deletion complex involved in the development of myeloid leukemia (16) and a region associated with a locus (*MODY*) which has been implicated in the development of non-insulin-dependent diabetes mellitus (17).

## MATERIALS AND METHODS

**Southern Blotting.** Ten micrograms of genomic DNA was digested with restriction enzymes, electrophoresed through agarose gels, and blotted to Gene-Screen (DuPont) using standard procedures (18, 19). Radiolabeled hybridization probes were prepared with the random hexamer-labeling technique (20). Repetitive sequences were reassociated with sheared genomic DNA before hybridization. Posthybridization filter washes were conducted under reduced stringency conditions (0.2× standard saline/citrate/0.1% SDS at 46°C) or high-stringency conditions (0.2× SSC/0.1% SDS at 68°C).

**Isolation of a Human Genomic Clone.** A *Sau3A* partially digested human genomic lambda library in EMBL3 was prepared and screened with a <sup>32</sup>P-labeled mouse agouti full-length cDNA probe that had been reassociated with human sheared genomic DNA. One positive clone was purified, and several restriction fragments were subcloned into

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Abbreviations: FISH, fluorescence *in situ* hybridization; FLpter, fractional length from the p terminal; *MODY*, maturity onset diabetes of the young; ORF, open reading frame.

the pGEM4 vector (Promega) for sequencing. Specifically, a 1.2-kb *Pst* I fragment, which hybridized to the mouse cDNA probe, but not total mouse DNA, was subcloned to generate the sequence homologous to the first coding exon. A 4.5-kb *Eco*RI fragment, which hybridized to two overlapping labeled 42-base oligonucleotides (5'-CACTGAACAAGAAA-TCCAAGAAGATCAGCAGAAAAGAAGCCG-3'; 5'-TTG-GAAGACCTCTCCGCTTCTCGGCTTCTTTCTGCT-GATC-3') complementary to the 5' or 3' ends of mouse exon III, respectively, with 25 bp overlapping in the middle, was subcloned and used to generate the sequence homologous to the second coding exon. A 6.2-kb *Eco*RI fragment, which hybridized to a probe containing mouse exon IV (called probe B in ref. 3), was subcloned and used to generate the sequence of the region homologous to the last agouti exon.

**DNA Sequencing.** Genomic subclones were sequenced by the Sanger dideoxynucleotide chain-termination method (21) using T7 DNA polymerase (United States Biochemical) (22). DNA sequence was analyzed by using the University of Wisconsin Genetics Computing Group sequence-analysis programs (23).

**Chromosomal Assignment.** For chromosomal assignment of the human agouti gene, PCR with 24 DNA samples from the NIGMS Cell Repository human-rodent somatic-cell-hybrid mapping panel 2 (24) and 3 samples from the human (NAIMR91), mouse (NA05862), and Chinese hamster (NA106588) parental cell lines, respectively, were done. An oligonucleotide (5'-CCTCTTACCATTACCCCTGA-3'), corresponding to flanking intron sequence 35 bp upstream of exon II and an oligonucleotide (5'-CTAGGTGACTTACCACAAT-3'), corresponding to the 3' splice junction of exon II, were used as primers A and B, respectively. PCR was done in 50- $\mu$ l volumes containing 100 ng of template DNA, 15 pM of primer A and B, 50 mM KCl, 10 mM Tris-Cl, 15 mM MgCl<sub>2</sub>, 0.01% (wt/vol) gelatin, 250 mM of each dNTP, and 1.4 units of *Taq* polymerase. Initial denaturation was at 95°C for 4 min, followed by 32 cycles, each consisting of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C. Ethidium bromide-stained PCR fragments were visualized after separation on a nondenaturing polyacrylamide gel.

**Mapping by Fluorescence *in Situ* Hybridization (FISH).** Prometaphase chromosomes for FISH analysis were obtained from cultured human lymphocytes following a slightly modified version of the method described by Rybak *et al.* (25). For regional mapping of the human agouti gene, the 17-kb genomic probe h20B1 was labeled with biotin-16-dUTP (Boehringer Mannheim) by nick translation. Labeling, hybridization, and signal detection was done as described (26, 27). The signal was amplified twice using biotinylated goat anti-avidin immunoglobulins (Vector). Slides were counterstained in ethidium bromide or 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) for band identification and were mounted in anti-fade solution containing 90% (vol/vol) glycerol/10% phosphate-buffered saline/phenylene diamine at 1  $\mu$ g/ml. A Zeiss axiophot fluorescence microscope ( $\times$ 63, filter LP420 and LP520) was used for analysis, and representative metaphases were photographed on Scotch T640 film. The map position of the agouti locus was determined by the assignment of FISH signals to G-bands and also by length measurements and its expression in terms of fractional length (FL) from the p terminus (pter) values (FL-pter) according to Lichter *et al.* (28).

**RNA Isolation and Reverse Transcriptase-PCR.** Poly(A)<sup>+</sup> RNA from normal human female subcutaneous adipose tissue was isolated using the FastTrack mRNA isolation kit with guanidinium-based lysis buffer (Invitrogen). Poly(A)<sup>+</sup> RNA from the remaining tissues was purchased from Clontech. Two hundred nanograms of poly(A)<sup>+</sup> RNA was reverse-transcribed to first-strand cDNA using random-hexamer primers and avian myeloblastosis virus reverse transcriptase

(Invitrogen). One-twentieth of the reaction mixture was then subjected to specific PCR amplification as follows. PCR was done for 1 cycle (94°C, 5 min), 35 cycles (94°C, 30 sec; 65°C, 1 min; 72°C, 1 min), and 1 cycle (72°C, 5 min) in 5% (vol/vol) glycerol with *Taq* I polymerase and 0.5  $\mu$ M 5' primer (5'-ATGGATGTCACCCGCTTACTCCTGGCC-3') and 3' primer (5'-GCGCTCAGCAGTTGAGGCTGAGCACGC-3'), which corresponds to the 5' or 3' ends of the putative open reading frame (ORF) of the juxtaposed exons on h20B1, respectively. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers (Clontech) were added to separate PCR reactions as a control for the same reverse transcriptase product. Amplified PCR products were subjected to electrophoresis on 1% agarose gel.

## RESULTS

**Isolation of a Human Clone.** To determine whether the agouti gene has been conserved in the human genome, the mouse cDNA clone was hybridized to Southern blots containing human genomic DNA. Reduced stringency hybridization conditions allowed for detection of cross-hybridizing DNA fragments with a variety of restriction enzymes (data not shown). Based on this result, the same mouse agouti cDNA probe was used to screen a human genomic library. This led to the isolation of a human genomic lambda clone called h20B1. Restriction mapping of this clone revealed that it contains an insert of  $\approx$ 17 kb.

Characterization of clone h20B1 revealed the presence of three distinct segments that each cross-hybridize with one of the three coding exons of the mouse agouti gene (Fig. 1). These cross-hybridizing regions were subcloned and subjected to nucleotide sequence analysis using primers derived from the sequences of the individual mouse exons. This analysis revealed that each cross-hybridizing region of h20B1 contains sequence that is homologous with one of the three coding exons of the mouse agouti gene. Additionally, each conserved region is flanked by consensus splice donor and acceptor sites, except for the last region which, as expected, is flanked only by a splice acceptor at its 5' end. A polypyrimidine tract and putative branch point signals 10–50 bp upstream of each conserved 3' splice acceptor site were also identified (data not shown).

Most notably, the ORF defined by the mouse agouti gene is retained within the individual blocks of conserved sequences in clone h20B1 (Fig. 1). In fact, juxtaposition of these conserved regions through the putative splice donor and acceptor sequences gives rise to an ORF of 396 bp. This ORF has the potential to encode a protein of 132 amino acids, which is very similar to the predicted 131-amino acid protein for the mouse. The ORF begins with a translation initiation codon (ATG) and ends with a stop codon (TGA) at positions nearly identical to that of the mouse (Fig. 1). In the region corresponding to exon IV, a single polyadenylation signal (AATATA) is located at a similar position to the AATAAT found in the mouse.

Comparison of the mouse and human regions indicates that the ORF sequences are 85% identical at the nucleotide sequence level (Fig. 1). In contrast, the human regions corresponding to the 5' and 3' untranslated portions of the mouse mRNA have diverged considerably (Fig. 1). The human sequence has a 3-bp deletion within the region corresponding to exon III and a 6-bp insertion in the coding region of sequences corresponding to exon IV. Both of the sequence differences are in increments of 3 bp, which preserve the evolutionarily conserved ORF. Overall, comparison of the deduced amino acid sequences shows that the predicted human protein is 80% identical to that in the mouse (85% homology with conservative changes) (Fig. 2). Like the mouse protein, the amino terminus of the putative human

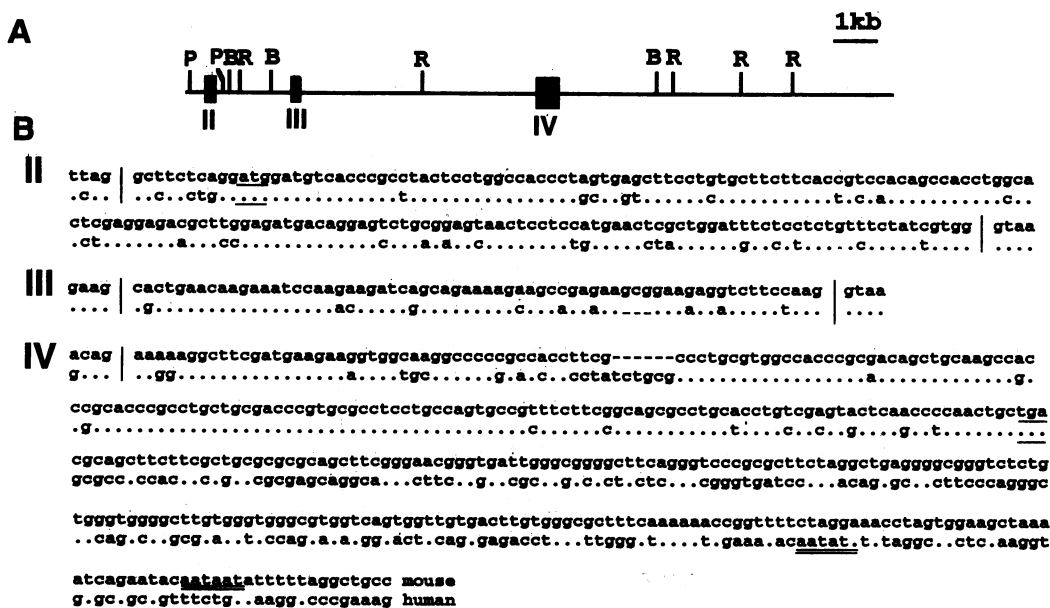


FIG. 1. (A) Genomic structure of human clone h20B1. Regions homologous to the three coding exons of the mouse gene are represented by black boxes and Roman numerals. P, *Pst* I; R, *Eco*RI; B, *Bam*HI. (B) Nucleotide sequence comparison of mouse and human exons of the agouti gene; the mouse sequence is shown above, and the human sequence is shown below. Identical nucleotides are represented as dots, different nucleotides are indicated, and an absence of nucleotides is noted as a dash. Actual exon sequences and the sequences of the 5' splice donors and 3' splice acceptors are separated by a space and vertical lines. The proposed translational initiation codons and stop codons are underscored by single lines, and the potential polyadenylation signals are underscored by double lines.

protein contains all of the features of a signal peptide, including a consensus cleavage site. The highly basic domain and the cysteine-rich domain near the carboxyl terminus (3)

Met	Asp	Val	Thr	Arg	Leu	Leu	Leu	Ala	Thr	Leu	Val	
.	.	.	.	.	.	.	.	.	.	.	Leu	
Ser	Phe	Leu	Cys	Phe	Phe	Thr	Val	His	Ser	His	Leu	
Val	.	.	.	.	.	.	Ala	Asn	.	.	.	
Ala	Leu	Glu	Glu	Thr	Leu	Gly	Asp	Asp	Arg	Ser	Leu	
Pro	Pro	.	.	Lys	.	Arg	.	.	.	.	.	
Arg	Ser	Asn	Ser	Ser	Met	Asn	Ser	Leu	Asp	Phe	Ser	
.	.	.	.	.	Val	.	Leu	.	.	Val	Pro	
Ser	Val	Ser	Ile	Val	Ala	Leu	Asn	Lys	Lys	Ser	Lys	
.	.	.	.	.	.	.	.	.	.	.	.	
Lys	Ile	Ser	Arg	Lys	Glu	Ala	Glu	Lys	Arg	Lys	Arg	
Gln	.	Gly	.	.	Ala	.	.	.	.	.	.	
Ser	Ser	Lys	Lys	Lys	Ala	Ser	Met	Lys	Lys	Val	Ala	
.	.	.	.	.	Glu	.	.	.	.	.	Val	
Arg	Pro	Pro	Pro	Pro	Ser	-	-	Pro	Cys	Val	Ala	
.	.	Arg	Thr	.	Leu	Ser	Ala	.	.	.	.	
Thr	Arg	Asp	Ser	Cys	Lys	Pro	Pro	Ala	Pro	Ala	Cys	
.	.	Asn	.	.	.	.	.	.	.	.	.	
Cys	Asp	Pro	Cys	Ala	Ser	Cys	Gln	Cys	Arg	Phe	Phe	
.	.	.	.	.	.	.	.	.	.	.	.	
Gly	Ser	Ala	Cys	Thr	Cys	Arg	Val	Leu	Asn	Pro	Asn	
Arg	.	.	.	Ser	.	.	.	.	Ser	Leu	.	
Cys	mouse											
.	human											

FIG. 2. Comparison of the amino acid sequence of the mouse agouti gene with that deduced from the homologous regions of human clone h20B1. Shown above is the reported mouse sequence (3); shown below is the human sequence as predicted from the ORF created by the juxtaposition of the regions from Fig. 1 that are homologous to the individual agouti exons. Identities are represented by dots, and differences are indicated; absences of amino acids are noted as dashes.

are also conserved between the predicted mouse and human proteins.

**Chromosomal Mapping.** Several genes linked to agouti in mouse chromosome 2 map to the long arm of human chromosome 20 (14, 15). In an attempt to determine whether clone h20B1 also maps to human chromosome 20, PCR was performed on 24 DNA samples from the NIGMS Cell Repository human-rodent somatic cell hybrid panel 2 (24). Primers for this experiment overlapped the region homologous to mouse exon II, and, as expected, gave rise to a 238-bp amplified fragment using human genomic DNA or DNA from the parental human cell line NAIMR91 as a template (data not shown). Analysis of the somatic-cell-hybrid panel 2 revealed that the agouti-specific 238-bp fragment was detected only in cell line NA10478 (data not shown), which is known to carry only human chromosome 20 in 84% of its cells and only chromosome 4 in another 8% of its cells (24). No specific products were amplified from the parental mouse line (NA05862), from the Chinese hamster cell line (NA10658), or from any of the other cell hybrids—particularly hybrid NA10115, which carries only human chromosome 4. These data unequivocally map clone h20B1 to human chromosome 20.

For regional localization experiments, FISH was done by using clone h20B1 as a probe. Hybridization was seen in the majority of metaphases in the proximal long arm of chromosome 20 (Fig. 3). Specific FISH signals were not seen on any of the other chromosomes. According to the banding pattern of 4',6-diamidino-2-phenylindole (DAPI)-stained metaphase chromosomes, the FISH signals for the agouti gene could be assigned to band 20q11.2. A few individual signals were detected on chromatids at other chromosomal sites in some metaphases, although their pattern was inconsistent; therefore, these signals were attributed to nonspecific background hybridization.

The position of h20B1 DNA on the physical FISH map of chromosome 20 (Fig. 3) was determined by length measurements from 13 selected chromosomes 20, and the result from this analysis were expressed in terms of FL-pter. The mean FL-pter value was determined to be 0.55 ± 0.05, which

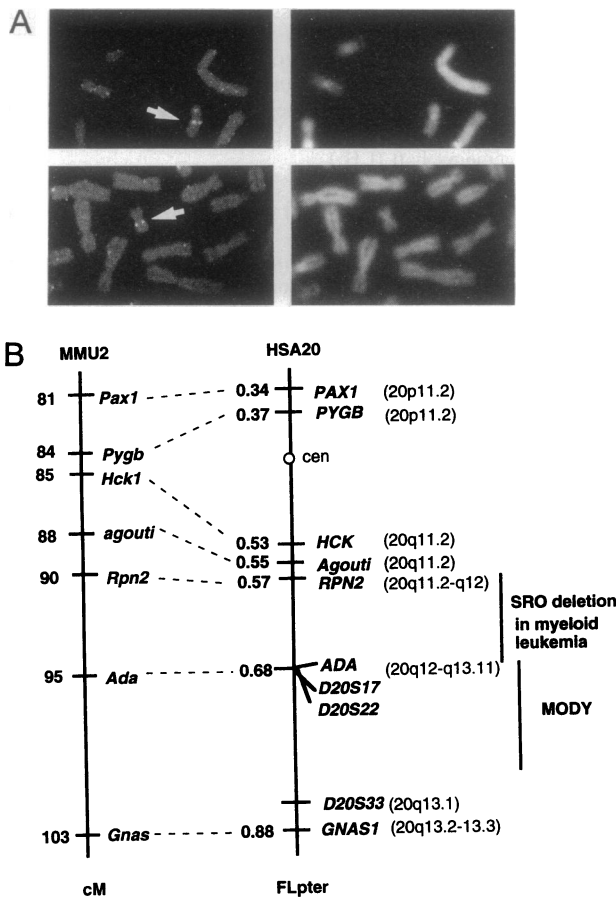


FIG. 3. (A) Assignment of clone h20B1 to human chromosome 20q11.2. Two partial metaphases (*Upper*, *Lower*) showing specific double signals by FISH at 20q11.2 (arrows) after hybridization with h20B1 DNA (*Left*) and the corresponding DAPI-fluorescence pattern of the same metaphases (*Right*). (B) Localization of the *agouti* gene within the physical FISH map of human chromosome 20 (HSA20) based on mean FL-pter values (15) demonstrating conservation of synteny within the respective segment on the composite linkage map of mouse chromosome 2 (MMU2) (14). Numbers to the left of MMU2 indicate genetic distances in centimorgans (cM) of the loci from the centromere. Numbers to the left of HSA20 indicate physical map positions of the loci as measured by FISH and expressed as FL-pter. The cytogenetic bands in which the human loci are located are indicated in parentheses at right of the chromosome. To the far right are indicated the locations on the cytogenetic map within which the *MODY* locus may lie based on genetic linkage data (17) and the regions of the chromosome commonly deleted in patients with myeloid leukemia (16).

corroborates the localization to band 20q11.2 (Fig. 3A). This assignment was also recently confirmed by FISH mapping of a 450-kb yeast artificial chromosome carrying the sequences hybridizing to h20B1. For this purpose, length measurements of 11 selected chromosomes 20 hybridized with this yeast artificial chromosome probe revealed an FL-pter value of  $0.55 \pm 0.04$ , which is essentially identical to that obtained with the h20B1 clone (data not shown).

**Expression in Human Tissues.** In an initial attempt to evaluate whether the homologous sequences on clone h20B1 are expressed, we used reverse transcriptase-PCR to assay for the presence of transcripts in a variety of adult human tissues, including three tissues that have been implicated in non-insulin-dependent diabetes mellitus (muscle, liver and fat). As shown in Fig. 4, the expected 403-bp *agouti*-specific fragment was detected only in adipose tissue and testis. To confirm that this 403-bp fragment actually arose from the corresponding human mRNA, the PCR fragment was puri-

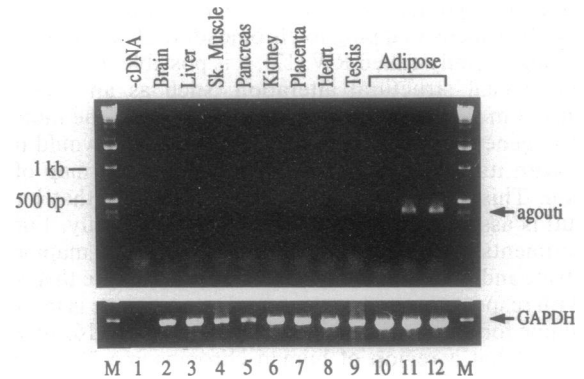


FIG. 4. Tissue distribution of human transcripts. (*Upper*) Amplified PCR products using *agouti*-specific primers and cDNA template reverse-transcribed from poly(A)<sup>+</sup> RNA of various tissues. Lanes: 1, no cDNA template in PCR reaction; 2–9, RNA from brain, liver, skeletal muscle, pancreas, kidney, placenta, heart, and testis, respectively; 10–12, RNA from adipose tissue of three different normal individuals. (*Lower*) Amplified PCR products using human glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-specific primers as a control.

fied, subjected to nucleotide sequence analysis, and found to contain the sequences within the mouse–human homologous regions in the h20B1 clone (data not shown). Moreover, sequence analysis of the 403-bp fragment also revealed that the three homologous regions were juxtaposed in a manner consistent with the utilization of the putative splice sites shown in Fig. 1 (data not shown). This result indicated that the homologous sequences in the h20B1 clone actually correspond to exons of a functional gene that is expressed in selected human tissues.

## DISCUSSION

Using a cross-species hybridization approach, we have isolated a human genomic clone that contains three evolutionarily conserved blocks of sequence, each of which correspond to one of the three exons that compose the entire coding region of the mouse *agouti* gene. The fact that the human clone contains a conserved ORF that is expressed in an RNA transcript and is predicted to encode a protein that is highly homologous and nearly identical in size to that of the mouse gene suggests that the conserved sequences from clone h20B1 actually correspond to the coding exons of a functional gene. Based on these findings, coupled with the mapping data, we propose that the evolutionarily conserved sequences in h20B1 actually correspond to the coding exons of the human homolog of the *agouti* gene.

FISH mapping experiments described here indicate that human *agouti* is closely linked to the *MODY* locus (Fig. 3). *MODY* is a form of non-insulin-dependent diabetes mellitus that has an early age of onset and an autosomal dominant mode of inheritance (29). Based on the fact that the dominant yellow *agouti* mutations in the mouse exhibit a non-insulin-dependent diabetic-like condition similar to that observed in *MODY* patients, we evaluated whether *agouti* was a candidate gene for *MODY* based on the close linkage between the two. Analysis of our comprehensive FISH data revealed that *agouti* maps to band 20q11.2, which is the region where the loci *HCK* and *RPN2* have been mapped (Fig. 3) (15). All three of these loci, *HCK*–*agouti*–*RPN2*, are located slightly more proximal to the 13-centimorgan sex-averaged interval encompassing *ADA*, *D20S17*, *PPGB*, *D20S16*, and *D20S75*, which is the region where *MODY* has been mapped (17). Therefore, taking into account only the genetic linkage data of *MODY* and our physical assignment of *agouti* to a slightly more proximal 20q segment (Fig. 3B), we would conclude that it

appears that agouti is unrelated to *MODY*. However, because the *MODY* locus mapping to chromosome 20 is based solely on a single family called RW (17), it is possible that a specific chromosomal structural alteration, such as an inversion, occurred in this family and that this feature of the mutation affects genetic recombination in a manner that would interfere with its accurate placement on a physical map of the region. This being the case, it is still possible that human agouti is associated with *MODY* in the RW family. Further experiments using microsatellite repeats, genetic mapping of the two, and mutation analysis will help to resolve this issue.

With respect to whether the human agouti gene is involved in tumor formation in humans, Roulston *et al.* (16) recently reported that a region of human chromosome 20q is commonly deleted in patients with myeloid leukemia. By cytogenetic and FISH analysis, a commonly deleted interstitial segment (SRO) mapping to 20q11.2–q12 was identified that contains the *ADA* and *SRC* loci and is flanked proximally by the ribophorin 2 gene locus (*RPN2*) and distally by *D20S17* (16). Our FISH results place the agouti gene close to the proximal boundary of this commonly deleted segment (16). Whether agouti maps within the deleted region is presently unclear, although, in the event that agouti occurs adjacent to the deleted region, a similar situation might exist to that seen in the dominant mouse mutation, lethal yellow (3, 13). Lethal yellow animals have an increased susceptibility to the development of both spontaneous and induced tumors. We recently determined that a 170-kb deletion in the region upstream of the agouti coding exons causes an activation of the gene and is likely responsible for the pleiotropic effects (13, 30).

Although the agouti pattern of pigmentation is evolutionarily conserved throughout many mammalian species, there has never been a published report describing human hair with the banded agouti-like pigmentation pattern. Therefore, the question arises as to what role, if any, the human agouti gene may have in pigmentation. The very strong sequence conservation within the coding exons of the gene would argue that the agouti gene has some functional role in humans. One possibility is that agouti has an identical function in humans and mice and that this includes signaling the melanocyte to produce pheomelanosomes. In this case, the lack of banded agouti pigmentation in humans could be related to the fact that humans have a continuous hair-growth cycle, which is different from the discontinuous molting hair growth cycles exhibited by various mammalian species that display agouti pigmentation. Alternatively, it is possible that the human and mouse agouti genes have evolved different regulatory mechanisms for expression.

Finally, based on our limited expression analysis of the human agouti gene, the production of agouti transcripts in adipose tissue from presumably normal individuals is provocative. In the mouse, it appears that agouti and other pigmentation genes exert their effects by regulating the level of cAMP within the melanocyte (31). If agouti regulates cAMP levels in the adipocyte in a similar manner, then the net effect of adipose-specific expression of agouti would be to decrease cAMP levels within the adipocyte. In this case, lower levels of cAMP would lead to decreased lipolysis due to inactivation of the hormone-sensitive lipase, which would contribute to adipocyte hypertrophy and obesity (5). The resulting obesity may clearly lead to insulin resistance (6) and possibly non-insulin-dependent diabetes (7). Therefore, in humans, agouti may have some functional role in the regulation of lipid metabolism within the adipocyte.

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