RESEARCH

Comparative Analysis of 1196 Orthologous Mouse and Human Full-length mRNA and Protein Sequences

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A large set of mRNA and encoded protein sequences, from orthologous murine and human genes, was compiled to analyze statistical, biological, and evolutionary properties of coding and noncoding transcribed sequences. Protein sequence conservation varied between 36% and 100% identity, with an average value of 85%. The average degree of nucleotide sequence identity for the corresponding coding sequences was also ~85%, whereas 5' and 3' untranslated regions (UTRs) were less conserved, with aligned identities of 67% and 69%, respectively. For some mouse and human genes, nucleotide sequences are more highly conserved than the encoded protein sequences. A subset of 32 sequences, consisting of only mouse/human protein pairs for which the human sequence represents a positionally cloned disease gene, had properties very similar to the larger data set, suggesting that our data are representative of the genome as a whole. With respect to sequence conservation, two interesting outliers are the breast cancer (*BRCA1*) gene product and the testis-determining factor (*SRY*), both of which display among the lowest degrees of sequence identity. The occurrence of both introns and repetitive elements (e.g., Alu, BI) in 5' and 3' UTRs was also studied. These results provide one benchmark for the "comparative genomics" of mice and humans, with practical implications for the cross-referencing of transcript maps. Also, they should prove useful in estimating the additional sampling diversity provided by mouse EST sequencing projects designed to complement the existing human cDNA collection.

The assertion that a pair of sequences is "highly homologous" or "strongly conserved" without reference to any objective scale of measurement is seen often in the genomics and general molecular biology literature. Quantifying the degree of similarity is important in any context, but this is true especially in conjunction with the automated analysis systems that are being developed to annotate the 100 million bases of human genomic sequence data that are anticipated over the next 2 years. Surprisingly, in this community little attention has been paid to studying the range of sequence variation between orthologous genes and proteins in different organisms, or among paralogous members of gene families in a single organism. This situation is made more urgent by the existence of >400,000 human cDNA sequences, and an additional 400,000 mouse cDNA sequences that are expected over the next

2 years (Washington University and Howard Hughes Medical Institute 1996)—there are already more than 50,000 mouse cDNAs in GenBank. Another important factor is the international effort to construct a transcript map of the human genome using gene-based reagents derived largely from cDNAs (Boguski and Schuler 1995) and the desirability of correlating this effort with a corresponding mouse gene map (M. Seldin and J. Nadeau, pers. comm.).

Much pioneering work has been done on the molecular evolution of mammalian genes (Nei 1987; Li and Graur 1991), and, as large portions of the mammalian genomic sequence become available during the next few years, many more biologists will be turning to these earlier studies as a foundation upon which to interpret the new data. Perhaps most pertinent to the current study is the work of Wolfe and Sharp (1993), who studied the nucleotide sequence conservation of 363 mouse and rat gene pairs representing 411 kilobases of sequence data. The current study addresses the issue of orthologous sequence conser-

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variation between 1196 mouse and human genes representing 2239 kilobases of sequence data. We studied the range of sequence variations in the 5' and 3' untranslated regions (UTRs) as well as the coding sequences (CDSs) and proteins. We examined some of the biological properties of those sequence pairs with extreme values of sequence conservation or divergence. We also analyzed sequence features such as the presence of introns and repetitive elements in mRNA untranslated regions, an issue of practical importance for anyone designing gene-specific sequence tagged sites (STSs) for use in mapping (Boguski and Schuler 1995). Our analyses yielded some surprising results that, upon further evaluation, are consistent with previous studies on the molecular evolution of rodents and humans. These findings are discussed in the context of interpreting human genomic and mouse cDNA sequences and also in terms of their implications for cross-referencing transcript maps of the mouse and human genomes.

RESULTS AND DISCUSSION

Nucleotide and protein sequences were aligned

as described in Methods. All results refer to aligned sequence pairs, excluding gaps. A table containing alignment length and sequence conservation for all 1196 mRNA and protein pairs is available as an electronic supplement on the World Wide Web at <http://www.cshl.org>. (Follow the links to this issue of the journal.)

Statistical Properties of the Data

The 878 5' UTR sequences consisted of 108,955 nucleotides. Alignment lengths ranged in size from 20 to 1521 nucleotides with a mean value of 124 nucleotides (standard deviation, SD = 127). These lengths were distributed narrowly, with 50% of the values in the range of 50–147 nucleotides; 90% of the values were <250 nucleotides (Fig. 1A). The 947 3' UTR sequences consisted of 397,040 nucleotides with alignment lengths of 40–3478 nucleotides. The mean and SD were 414 and 426 nucleotides, respectively. The 3' UTR lengths were distributed somewhat more broadly than the 5' UTR lengths, with 50% of the values

between 133–558 nucleotides; 90% of the values were <942 nucleotides (Fig. 1D). Nevertheless, in both cases a number of outlying values are apparent. For example, the two longest 5' UTRs are those of mouse beta-2-adrenergic receptor (2211 bases; GenBank accession no. X15643) and a human growth/differentiation factor (1346 bases; GenBank accession no. M62302). For 3' UTRs, the longest were those of the human tissue inhibitor of metalloproteinase-3 (3663 bases; GenBank accession no. U14394), the human recombination-activating protein RAG-1 (3301 bases; GenBank accession no. M29474), and a human stem-cell leukemia gene product (3427 bases; GenBank accession no. M61108). On average, 3' UTR alignments were 3.34 times longer than 5' UTR alignments, and this obser-

Figure 1 Scatter plots of sequence alignment length vs. sequence conservation. (A,B,D) Aligned nucleotide sequence identity on the x-axis. (C) Aligned protein (AA) sequence identity.

vation is consistent with previous results on the size distributions of untranslated regions from human mRNAs (Pesole et al. 1994).

The average aligned identity of human/mouse 5' UTRs is 67.47% (SD = 13.2), and the average aligned identity of human/mouse 3' UTRs was similar at 69.13% (SD = 12.4). For both 5' and 3' UTRs, the degrees of sequence conservation were distributed broadly between 30–40% and 100% identity (Fig. 1A,D; Fig. 2).

The 1196 CDSs consisted of 1,733,644 nucleotides. Alignment lengths ranged in size from 135 to 13,638 nucleotides with a mean value of 1450 nucleotides (SD = 1194). The corresponding encoded proteins had alignment lengths from 44 to 4545 amino acids with a mean and SD of 481 and 397 residues, respectively. The ratio of average nucleotide-alignment-length to average protein-alignment-length is ~3, as it should be, given the triplet nature of the genetic code. The distributions of CDS and protein sizes are quite narrow (Fig. 1B,C). At least 50% of aligned CDSs fall between 758 and 1767 nucleotides, and 90% are <2669 nucleotides in length. For aligned proteins, 50% are in the range of 249 and 587 amino acid residues, and 90% are <876 residues in length. Outlying values in these size distributions include the sequences of the human

LDL receptor-related mRNA/protein (14,896 bases, 4544 residues; GenBank accession no. X13916), human heparin sulfate proteoglycan (14,327 bases, 4391 residues; GenBank accession no. M85289), and the Duchenne muscular dystrophy gene product, dystrophin (13,957 bases, 3685 residues; GenBank accession no. M18533).

The average aligned identity of human/mouse CDSs is 84.62% (SD = 6.78) and the averaged aligned identity of human/mouse proteins is very similar at 85.39% (SD = 12.62). Sequence conservation is distributed more narrowly for coding sequences compared with proteins (Figs. 2 and 3). Twenty-seven proteins were 100% identical in sequence between humans and mice (Table 1). At the other extreme, some human/mouse protein pairs were as little as 36–41% identical (Table 2).

Biological and Evolutionary Aspects

Although the 5' and 3' untranslated regions of

Figure 2 Box plots of aligned sequence identity distributions. For each category, the central box depicts the middle half of the data between the 25th and 75th percentiles; the black dots indicate the medians of each distribution. Extreme values in each distribution are indicated by the circles which fall outside the main body of data.

Figure 3 Distributions of sequence conservation in aligned mRNA coding sequences and the proteins they encode.

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Table 1. Human and Mouse Proteins with Identical Amino Acid Sequences

Protein name ^a	Human seq. acc. number	Mouse seq. acc. number	CDS length ^b	CDS identity (%) ^c
BTG1	X61123	L16846	516	96.71
Calmodulin	M19311	X61432	450	84.01
DNA-binding protein (insulinoma rig-analog)	J02984	M33330	438	89.95
Enteric smooth muscle gamma-actin	X16940	M26689	1131	92.48
F-actin capping protein beta subunit	U03271	U10407	819	92.67
GTP-binding protein (RAB1)	M28209	Y00094	618	95.47
GTP-binding protein G25K	M57298	L11318	573	95.64
GTP-binding protein (rac)	M29870	X57277	579	91.36
Histone H3	X00090	X16496	411	86.13
Histone H4 (human gene H4/e)	X60484	X13235	312	89.74
Histone H4 (human gene H4F2)	M16707	V00753	312	87.50
Lupus autoantigen (snRNP)	J04615	X62648	723	91.15
MHC protein hom. to chicken B complex prot.	M24194	X75313	954	90.57
Myelin proteolipid protein	M17085	M15442	729	96.84
Nerve-terminal protein (isoform SNAP-25b)	L19761	M22012	621	94.69
Nucleic acid binding protein—hnRNP-E2	X78136	L19661	1086	97.61
Phosvitin/casein kinase II beta subunit	X16312	X56502	648	93.67
PPP1CB	X80910	M27073	984	94.61
Pre-mRNA splicing factor (SRp20)	L10838	X53824	495	91.92
Ribosomal protein L30	X79238	K02928	348	93.97
Ribosomal protein L32	X03342	K02060	408	91.42
Ribosomal protein L37a	L06499	X73331	279	90.68
Ribosomal protein S4	M58459	M73436	792	91.04
Ribosomal protein S8	X67247	X73829	627	89.79
Ribosomal protein S24	D31520	X60289	393	89.06
Ribosomal protein S28	L05091	U11248	210	87.62
Skeletal muscle alpha-actin	J00068	M12866	1134	90.74
Thymosin beta-4	M17733	X16053	135	93.33

^aProtein names and synonyms are taken from GenBank or SwissProt records and the accession numbers refer to nucleotide sequences in GenBank.

^bLength of the sequenced alignment between the mouse and human mRNA coding sequences.

^cNucleotide sequence conservation.

eukaryotic mRNAs have important biological functions (Jackson 1993; Gray and Hentze 1994; Decker and Parker 1995) it has been known for some time that their sequences are under less selective pressure than sequences that encode proteins (Nei 1987; Li and Graur 1991). In our data set, the 5' and 3' UTRs are 31–33% divergent between human and mouse, whereas CDSs show only an ~15% difference in aligned nucleotide identity (Fig. 2). The degree of sequence divergence was essentially identical (15%) for CDSs and the proteins they encode (Fig. 2). This was somewhat surprising given the usual assumption that the detection of homology is much more sensitive using protein sequences rather than their cognate mRNAs (Doolittle 1981). Although

this assumption is certainly true in the context of homologous genes from very distantly related organisms (e.g., humans and yeast; Tugendreich et al. 1994), it may not be true for mammalian species that shared a common ancestor *only* 80–100 million years ago.

Even though the mean values of sequence conservation of CDSs and proteins are nearly identical, the aligned amino acid identities show considerably greater variance ($\sigma^2 = 159$) than aligned nucleotide identities ($\sigma^2 = 46$) (Figs. 2 and 3). Also, the distribution of aligned amino acid identities is skewed toward higher values with a large number between 95% and 100% identity (Fig. 3). In contrast, the distribution of aligned nucleotide identities shows very few

Table 2. The 25 Most Divergent Human and Mouse Proteins

Protein name	Human seq. acc. number	Mouse seq. acc. number	5' UTR identity (%)	CDS identity (%)	Protein identity (%)	3' UTR identity (%)
Alpha1-acid glycoprotein (orosomucoid)	M13692	M27008	62.79	63.37	48.76	51.25
B lymphocyte activation antigen B7 (Ig)	M27533	X60958	63.88	60.74	41.11	55.38
brk/Srm tyrosine kinase	X78549	D26186	50.00	55.76	46.40	50.25
Centromere protein C (CENP-C)	M95724	U03113	61.81	70.95	52.44	77.36
CTLA4 counter receptor	U04343	L25606	52.43	64.25	49.20	51.44
Fas (APO-1)	X63717	M83649	54.72	66.46	49.54	61.47
Interferon beta	M28622	X14455	65.71	68.98	50.00	66.83
Interferon gamma receptor	J03143	M28995	55.26	68.60	52.26	55.14
Interferon-gamma induced protein	M63838	M31419	67.92	67.77	49.30	66.11
Interleukin 6	X04430	J03783	76.83	61.77	41.43	74.03
Interleukin 4	M13982	M25892	77.53	51.31	43.57	84.85
Interleukin 6 receptor	X12830	X53802	55.48	69.99	53.17	62.18
Interleukin-4 receptor	X52425	M27959	51.43	66.49	52.36	59.29
Kappa casein	M73628	M10114	77.78	63.45	36.10	71.76
Leukosialin (sialoporphin)	J04168	S70677		62.03	48.96	50.76
NKR-P1A (lectin superfamily)	U11276	M77677	57.78	63.23	46.40	
Olfactory receptor	X64995	M84005		56.10	42.72	
Prolactin-inducible protein (PIP)	J03460	J02510		59.54	45.83	
Proteoglycan core protein	X17042	X16133	58.33	69.78	50.30	67.76
RNA polymerase I-specific termination factor	X83973	X83974	50.00	69.83	51.90	48.20
Sry (sex determining region of Y-chromosome)	L08063	U03645		64.89	41.61	
T-cell glycoprotein Lyt-3	X13445	M17534	45.28	70.72	52.40	51.10
T-lymphocyte surface CD2 (T11) antigen	M14362	M18934		68.44	52.34	68.75
Tcp-10	U03399	X58170	62.34	64.55	50.92	34.32
Tissue factor-membrane protein that initiates the blood clotting	J02931	M26071	87.68	68.17	51.04	71.15

These sequences represent many of the outlying values indicated as circles in Fig. 2. Sequence names, accession numbers, and aligned sequence conservation are as described in Table 1.

cases in the 95–100% range, but there is an obvious peak between 85% and 90% identity. We interpret these differences in the shapes and spreads of the distributions as a reflection of the rapid accumulation of nucleotide changes in synonymous (third base) codon sites followed by a slower mutation rate in nonsynonymous sites (Li and Graur 1991). Wolfe and Sharp (1993) have examined this phenomenon in a sample of 363 orthologous mouse and rat genes with the interesting finding that, in CDSs encoding less well-

conserved proteins, aligned nucleotide identities exceeded aligned amino acid identities and that the “cross-over point” was ~93%. In our data set of mouse and human genes, the cross-over point lies at 84–85% (Fig. 4). This lower level undoubtedly reflects the more ancient divergence time of humans and mice (80 million years ago; Novacek 1992) compared with mice and rats, which diverged only 15 million years ago (Flynn et al. 1985).

To determine if any interesting biological de-

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Figure 4 Scatter plot of protein vs. nucleotide sequence conservation. The dashed line represents a linear regression model of the data. The solid line allows the estimation of the crossover point between nucleotide and protein sequence identity. This is seen more easily in the inserted plot, which shows a more detailed picture of this region.

tails of protein function or evolution lay hidden in the distribution of aligned sequence identities, we considered various subsets of the mouse/human sequence pairs. First, we examined extreme values at the upper and lower tails of the distribution. There were 27 proteins that were identical in sequence between human and mouse (Table 1) and these include several well-known cases of very slowly evolving genes, for example, actins and histones H3 and H4 (Nei 1987; Li and Graur 1991). Also, a number of ribosomal proteins are conserved 100%, presumably because the ribosome is a complex structure of interacting proteins where even small alterations in protein conformation are not tolerated. Several *ras*-like GTPases, a variety of other signal transduction proteins, and some DNA- and RNA-binding proteins are also evident in this list.

The 25 least-conserved proteins range from 36% to 52% identical and are shown in Table 2. It is interesting to note that for several cases, the 5' and 3' UTRs are more highly conserved than the coding sequences. As to the biological nature of these 25 least-conserved proteins, Murphy (1993) has pointed out previously that proteins involved in antimicrobial host defenses (e.g., interleukins, interferons) are among the most diver-

gent of proteins, and several examples of such are present in our sample (Table 2). Interferons have been noted independently to be very rapidly evolving proteins (Li and Graur 1991). In the original papers describing 19 of these 25 proteins, the similarity between the mouse and human sequences was explicitly discussed, and in five cases the authors describe the similarity as high (Yokota et al. 1986; Murphy et al. 1987; Gubbay et al. 1990; Watanabe-Fukunaga et al. 1992; McKay et al. 1994); in only a single case was the similarity (appropriately) described as low (Munro and Maniatis 1989).

Two important questions about sequence pairs at the least-conserved end of our identity distribution are: (1) How can we be sure that these are true orthologs given the low degree of aligned identity; and (2) how can we be sure that this sample is representative of the spectrum of protein conservation between the mouse and human genomes as a whole? Regarding the first question, protein pairs were selected carefully in the context of sequence phylogenies where orthologous genes can be distinguished from paralogous genes. In some cases homology of synteny can be involved to support the claim (see below). Regarding the second question, has our data set been subject to ascertainment bias resulting from technical aspects involved in the original cloning of the genes? For example, if a human gene is cloned first and we are seeking the corresponding mouse gene by hybridization screening (or vice versa), are we likely to isolate only genes that are relatively well-conserved and, thus, bias the data away from more divergent orthologs? To address this question, we examined the original cloning papers and found that 12 out of the 25 most-diverged mRNA sequences (Table 2) were isolated and sequenced in independent projects and noted to be orthologous only after the fact. In 12 of the remaining cases, human or mouse cDNA libraries were screened using a sequence from the other species as a probe, and in the last case, a rat sequence was used to identify both the mouse and human genes. In four cases, the orthologous nature of the sequences was confirmed by syntenic human/mouse mapping data (Davies and Willson 1991; Goirda and Trucco 1991; Saitoh et al. 1992; Behlke et al. 1993; Islam et al. 1993; Coward et al. 1994; Lanier et al. 1994; McKay et al. 1994).

Rates of nucleotide substitution in fourfold degenerate sites in human and rodent coding sequences are ~ 3.33 substitutions per site per 10^9

Table 3. Similarities between Human Inherited Disease Genes Identified by Positional Cloning and Their Mouse Orthologs^a

Disease	Gene symbol	Gene product	Human seq. acc. number	Mouse seq. acc. number	CDS identity (%)	Protein identity (%)
Aarskog-Scott syndrome	FGD1	rho/rac GEF	U11690	U22325	91.7	93.8
Achondroplasia	FGFR3	fibroblast growth factor receptor-3	M58051	S56291	85.7	93.4
Adenomatous polyposis coli	APC		M74088	M88127	87.6	90.6
Amyotrophic lateral sclerosis	SOD1	Cu-Zn superoxide dismutase	X02317	M35725	83.9	83.8
Aniridia	PAX6	oculorhombin	M77844	M77842	92.9	99.3
Breast cancer type1	BRCA1		U14680	U32446	75.2	57.2
Chronic granulomatous disease	NCF1	neutrophil cytosol factor 1	M55067	L11455	80.9	81.7
Congenital adrenal hyperplasia	CYP21	steroid 21-mono-oxygenase	M26856	M15009	77.4	72.4
Cystic fibrosis	CFTR	transmembrane conductance regulator	M28668	M69298	81.4	78.5
Diastrophic dysplasia	DTD	sulfate transporter	U14528	D42049	83.4	80.9
Duchenne muscular dystrophy	DMD	dystrophin	M18533	M18025	87.8	86.9
Fragile-X syndrome	FMR1		S65791	L23971	95.7	96.6
Glycerol kinase deficiency	GK	glycerol kinase	L13943	D16102	91.7	97.3
Gonadal dysgenesis	SRY	testis-determining factor	L08063	U03645	64.9	41.6
Hereditary non-polyposis colon cancer	hPMS2	DNA mismatch repair enzyme	U14658	U28724	80.8	73.4
Hereditary non-polyposis colon cancer	hMSH2	DNA mismatch repair enzyme	U04045	X81143	86.7	92.5
Huntington's disease	HD	huntingtin	L12392	L28827	86.4	91.18
Hyperexplexia	GLYR A2	inhibitory glycine receptor	X52009	S73717	90.5	97.8
Menkes disease	MNK	heavy metal binding protein	X69208	U03434	85.7	84.4
Miller-Dieker lissencephaly	PAF	acetylhydrolase subunit	L13385	L34659	95.2	99.76
Multiple endocrine neoplasia 2A	RET	receptor tyrosine kinase	M57464	X67812	84.1	85.3
Myotonic dystrophy	DM	mutonin protein kinase	L19268	Z21503	83.1	83.6
Neurofibromatosis type 1	NF1	neurofibromin	M89914	X54924	91.5	99.5
Neurofibromatosis type 2	NF2	merlin	L11353	L27090	90.0	98.5
Norrie disease	NDP		X65882	X83794	91.9	95.4
Retinoblastoma	RB1		M15400	M26391	88.6	89.6
Thomsen disease	CLC1	skeletal muscle chloride channel	Z25884	X62896 ^b	88.4	91.5
vonHippel-Lindau syndrome	VHL		L15409 ^b	U12570	84.4	90.1

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Table 3. (Continued)

Disease	Gene symbol	Gene product	Human seq. acc. number	Mouse seq. acc. number	CDS identity (%)	Protein identity (%)
Waardenburg syndrome	PAX3	paired box homeo-domain protein	U02309 ^b	X59358	91.5	97.9
Wilms tumor	WT1	zinc-finger protein	X51630	M55512	90.8	96.7
Wilson disease	WND	copper-transporting ATPase	U03464	U38477	66.3	57.4
X-linked adrenoleukodystrophy	ALD		Z21876	Z33637	86.1	91.4

^aA complete list of such genes may be found at http://www.ncbi.nlm.nih.gov/dbEST/dbEST_genes/ where each is linked to phenotype and other information in Online Mendelian Inheritance in Man (OMIM).

^bCoding sequence is not full-length.

years (Li and Graur 1991), or 0.53 substitutions per site over the elapsed time since the mammalian radiation, ~80 million years ago. Applying the Jukes and Cantor (1969) model to correct for multiple substitutions, the observed divergence is expected to be 0.38. It follows that, in the absence of selective pressure, the most divergent mouse and human DNA sequences should be 62% conserved (38% diverged). The fact that we have some values even lower than this in our data set (Fig. 4 and Table 2) suggests that we do indeed have an adequate sample of the most divergent mouse and human protein pairs.

A definitive answer to the question of how representative is the data at the lower end of the distribution may have to await the acquisition of large amounts of syntenic human and mouse genomic sequence in which random samples of protein-coding genes are found. Current samples of orthologous human and rodent genomic DNA have shed some light on patterns of conservation in noncoding sequences (Koop and Hood 1994), but have not yet extended our knowledge of rapidly evolving sequences that encode proteins. However, we can extrapolate the lower level of detectable orthology from a consideration of mutation rates in fourfold degenerate codon sites (Li and Graur 1991) and also from a linear regression analysis of our nucleotide and amino acid alignment data (Fig. 4).

Another interesting conclusion from our data is that, if we extrapolate to even lower degrees of sequence identity than those present in our sample of aligned sequences, we can see that at the level of 40% identity between mouse and

human CDSs, no identical residues at all are expected in the protein alignments (Fig. 4). Of course, this is an oversimplified view of protein evolution, and, in practice, amino acid substitution matrices are used to detect distant similarities and assess their significance (Henikoff and Henikoff 1993). As a point of reference, even the most distantly related sequence pair in our study (kappa casein; Table 2) yields unquestionably significant similarities between the mouse and human orthologs in the context of a data-base search using the default (BLOSUM62) scoring matrix (BLASTN score = 665; p value = 5.9×10^{-58} ; BLASTP score = 231; p value = 1.9×10^{-33}).

To date, there have been >60 human disease genes isolated by positional cloning (Bassett et al. 1996). We have found that in 32 cases the orthologous mouse sequence is available also (Table 3). In many ways, the properties of these sequences recapitulate the characteristics of our larger mouse/human collection. They range in size from small, globular enzymes such as superoxide dismutase (153 residues) up to the 3785-residue structural protein, dystrophin. Whereas the mean values of sequence conservation for DNA and protein alignments in our large set of mouse/human homologs are 84.62% and 85.39% identity, respectively, the mean values of sequence conservation for the disease gene coding sequences and proteins are very similar at 86.0% and 87.1%, respectively. Because these disease genes were cloned based solely by map position, and yet are so similar in sequence properties to our larger, 1196-sequence sample, this supports

our contention that the latter sample is not subject to serious ascertainment bias based on technical aspects of functional cloning.

Last, the list of disease genes contains several outliers with respect to sequence conservation. These include the breast cancer (*BRCA1*) gene product (57.2% identical) and the copper-transporting ATPase that is the product of the Wilson disease gene (57.4% identical). The testis-determining factor, *SRY*, is even more divergent (41.6% identical between mouse and human). In our data set of 1196 proteins, only kappa casein (Table 2) is more divergent than the *SRY* gene product. Rapid sequence divergence may be important for the function of these proteins.

Introns and Interspersed Repeats

We have examined our data set for the occurrence of introns and repetitive elements. Such information has practical implications for gene mapping (Wilcox et al. 1991) and cloning (Yulug et al. 1995).

To determine the relative frequency of introns in 5' and 3' UTRs, we performed a text query search of sequence feature annotations in GenBank records. Also, BLASTN searches were done using 5' and 3' UTR sequences from our data set as queries against a genomic sequence subset of the primate and rodent divisions of GenBank. In our data set, 954 mRNAs had 5' UTR sequence in either mouse or human sequence records. Among these, 58 5' UTRs contained one or two introns, although there are two human mRNA sequences with as many as four introns annotated in the GenBank record (accession nos. U19251 and L22214). In our data set, 1077 mRNAs had 3' UTR sequences and only seven of them had one or two introns. In all but one of these cases the presence of the 3' UTR intron was confirmed by the comparison of the mRNA sequences with the genomic one. Thus, introns are about nine times less likely to be found in 3' UTRs compared with 5' UTRs. We cannot determine the absolute frequencies of introns in untranslated regions from these data because corresponding genomic sequence data exists for only a fraction of the mRNA sequences in our collection.

In the 3' UTRs, introns usually are close to the stop codon. The closest intron is in position 13/14 at the human growth hormone-dependent, insulin-like growth factor-binding protein mRNA (GenBank accession no. M31159). The

most distant intron is located at position 89/90 of the human antigen CD36 3' UTR (GenBank accession no. M98399).

Previously, Yulug et al. (1995) have studied the frequency and positions of *Alu* repeats in a large collection of human mRNAs and found them to be present in 5% of fully spliced sequences, with the majority of cases occurring in the 3' UTR. Our analysis includes mouse as well as human mRNAs and a more extensive collection of different types of repeats.

The most abundant group of mammalian repeats are short interspersed repetitive elements (SINEs), and members of this group are found most frequently in our collection of sequences. We found 67 *Alu* sequences in 57 human 3' UTRs, or in 5.7% of the 1077 3' UTRs in our data set (a frequency similar to that observed by Yulug et al. 1995). Our human 3' UTR sequences also contained 18 mammalian-wide interspersed repeats (MIRs), three fragments of L1 [long interspersed repeated sequences (LINE)] repetitive elements, seven different medium reiterated frequency repeats (MERs), four repeat fragments of retroviral origin, and one fragment of a transposon-like human DNA transposons related to the *Drosophila* transposon pogo (tigger) repeat (Jurka et al. 1992; Smit and Riggs 1996). Similarly, most of the repeats found in mouse 3' UTRs are SINEs: 42 B1 or B2 elements were observed. Additionally, we found five fragments of the mouse L1 retroposon and 21 fragments of other mouse retroelements. In summary, 91 (8.5%) of human 3' UTR sequences and 70 (3.7%) of mouse 3' UTR sequences showed evidence of some type of repetitive element.

In contrast, the frequency of repetitive elements in 5' UTRs is much lower. In our set of 954 human 5' UTRs, there were only 10 *Alu*, three L1 elements, four MIRs, and seven fragments of other transposon-like repeats. In mouse 5' UTRs only five B1 retroposons, one B2 element, and three fragments of other repeats were observed. Thus, repetitive elements are three to seven times more likely to be found in 3' UTRs compared with 5' UTRs.

In one case, we observed MIR insertions at orthologous positions in the 3' UTRs of both mouse and human interleukin-10 mRNAs. The elements are 65.4% identical and reside 1423 bases downstream of the stop codon in mice and 1570 bases downstream of the stop codon in humans. The IL-10 MIR element is a molecular fossil of a retrotransposition event that occurred ~150

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million years ago (assuming a constant mutation rate, and an eutherian radiation date of 80 million years ago).

Implications for the "Comparative Genomics" of Mice and Humans

A complete description of the comparative genomics of two organisms would include pairwise sequence alignments of genomic DNA from all homologous regions among their chromosomes. We are a long way from that goal for humans and mice. Nevertheless, the human/mouse comparative map is quite extensive, currently containing 1416 loci in 181 conserved linkage groups (DeBry and Seldin 1996). Furthermore, a glimpse of comparative genomic structure, at the ultimate resolution of nucleotides, is available through a small number of sequence contigs that exist for both species (Koop 1995; Lamerdin et al. 1995). The present study supplements this information by providing an analysis of ~1200 functionally cloned, transcribed, protein-coding sequences. Here we have computed the simplest, most practical, and reproducible measure of sequence conservation, namely, percent identity after optimal alignment. Although we avoided using BLASTN *p*-values as a measure, because of their dependence on the size of the data base and scoring matrix used, the availability of our complete data set on the World Wide Web will allow anyone to compute the measure most suitable for their specific purposes. Currently, we are performing synonymous and nonsynonymous rate calculations on the coding sequences in our data set, and these results will be reported elsewhere.

Our results on the distributions and averages of pairwise sequence similarities between mouse and human mRNAs should prove useful in interpreting mouse cDNA sequencing data (Washington University and Howard Hughes Medical Institute 1996). One of the principle goals of this project is to supplement the human cDNA collection to produce a more comprehensive sample of mammalian genes. Thus, it will be very important, when aligning a mouse cDNA to a human gene, a human cDNA to a mouse gene, or human and mouse cDNAs to each other, to have some standard of comparison for distinguishing between orthologous sequences and paralogous sequences, that is, different members of a multi-gene family.

Using the alignment information produced in our study, it should also be possible to develop

sequence-based mapping reagents that may be used for physical and genetic mapping in either species. Such reagents could extend the human/mouse comparative map, but first it will be necessary to correlate our results, which are based upon sequence alignments, with results based upon other criteria (O'Brien and Graves 1991). Human/mouse homology maps, based upon these criteria, are available through the Mouse Genome Database at the Jackson Laboratory, Bar Harbor, ME (<http://www.infomatics.jax.org/>).

Finally, our collection of 1196 orthologous mouse and human genes might be useful as a standard for more extensive cross-referencing among other, more distantly related genomes such as those of nematodes and yeast (Bassett et al. 1995).

METHODS

Human and murine mRNA sequences were extracted from GenBank Release 87 (March 1995). Orthologous sequences were distinguished from paralogous sequences using the molecular phylogenies provided in the HOVERGEN data base of homologous vertebrate genes (Duret et al. 1994). Of the 3716 protein families in Release 11 of HOVERGEN, 1236 correspond to mRNA sequences with full-length coding regions available for both mouse and human species. Each of the 1236 phylogenetic trees was inspected and orthologous pairs of genes selected. This was a crucial step in assembling data appropriate for further study because some of the families contain subfamilies with different human/mouse orthologs and other families include full-length human and mouse coding sequences but situated on different branches of a phylogenetic tree, indicating a paralogous origin of these sequences. Whenever a given protein was represented by several GenBank records, the longest possible mRNA sequence was chosen for the human/mouse comparison. This set of HOVERGEN-derived sequences was supplemented by 32 mRNA/protein sequences corresponding to positionally cloned human disease genes (Table 3), that appeared subsequent to the release of HOVERGEN 11 and for which mouse homologs were available. These human/mouse pairs were identified through literature and homology searches.

In total, there were 1196 orthologous human/mouse mRNA pairs included in this study. A number of these mRNAs had either very short, or unknown, 5' and 3' UTRs. We excluded all 5' UTRs <20 nucleotides and all 3' UTRs <40 nucleotides from the study. Consequently, 878 5' UTRs and 947 3' UTRs met these minimum-length criteria.

Human and mouse mRNA and protein sequences were aligned using the *sim2* program (Chao et al. 1995) that, by sequence accession number, extracts sequence data directly from GenBank (Benson et al. 1996) using the *Entrez* application programming interface (ftp://ncbi.nlm.nih.gov/toolbox/ncbi_tools). This assures that the most recent data always was used. The *parsesim* program (J. Zhang, unpubl.) was used for the extracting the different mRNA regions (coding and untranslated se-

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quences) and protein sequences from ASN.1 files into the separate text files. The final alignments of both nucleotide and protein sequences were computed using the *gap* program that utilizes a global, optimal alignment algorithm (Huang 1994).

The entire set of human and mouse mRNA sequences was searched for different repetitive elements using the CENSOR program (Jurka et al. 1996). The program was run in the "conservative" mode against a reference collection of rodent or human repeats (Jurka 1995).

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