Mammalian Overlapping Genes: The Comparative Perspective

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It is believed that 3.2 billion bp of the human genome harbor ~35,000 protein-coding genes. On average, one could expect one gene per 300,000 nucleotides (nt). Although the distribution of the genes in the human genome is not random, it is rather surprising that a large number of genes overlap in the mammalian genomes. Thousands of overlapping genes were recently identified in the human and mouse genomes. However, the origin and evolution of overlapping genes are still unknown. We identified 1316 pairs of overlapping genes in humans and mice and studied their evolutionary patterns. It appears that these genes do not demonstrate greater than usual conservation. Studies of the gene structure and overlap pattern showed that only a small fraction of analyzed genes preserved exactly the same pattern in both organisms.

[Supplemental material is available online at www.genome.org and http://posnania.cbio.psu.edu/research/overlapping_genes.html.]

Overlapping genes occur frequently in viral and cellular prokaryotic genomes as well as in organelles such as mitochondria (Normark et al. 1983). Until recently, it was believed that they occur much less frequently in eukaryotic nuclear genomes. Although their presence in human and other species' genomes was reported previously (Williams and Fried 1986; Lazar et al. 1989; Miyajima et al. 1989; Burke et al. 1998; Cooper et al. 1998; Bachman et al. 1999; Shintani et al. 1999; Misener and Walker 2000; Morelli et al. 2000; Slavov et al. 2000; Zhuo et al. 2001), until lately very little was known about their frequency and genome-wide distribution. Recent reports show that overlapping genes occur relatively frequently in human and other mammalian genomes (Kiyosawa and Abe 2002; Lehner et al. 2002; Okazaki et al. 2002; Shendure and Church 2002; Yelin et al. 2003). Nevertheless, there is still little known about the origin, evolution, or cross-species conservation of overlapping genes.

Shintani et al. (1999) suggested that the overlap between two genes studied by them, *ACAT2* and *TCP1*, arose during the transition from therapsid reptiles to mammals, and that the overlap could have happened in one of two ways. In one scenario, the rearrangement may have been accompanied by the loss of a part of the 3′-untranslated region (UTR), including the polyadenylation signal, from one gene. By chance, however, the 3′-UTR of the new neighbor on the opposite strand contained all the signals necessary for termination and transcription so that the translocated gene could continue to function. Alternatively, the two genes became neighbors through the rearrangement but at first did not overlap. Later, one of the genes lost its original polyadenylation signal, but was able to use a signal that happened to be present on the noncoding strand of the other gene. Keese and Gibbs (1992) suspect that overlapping genes arise as a result of overprinting—a process of generating new genes from pre-existing nucleotide sequences. However, both studies were done based on a single pair of eukaryotic overlapping genes. The hypothesis by Shintani et al. (1999) can only be applied to those overlapping gene pairs in which the overlap occurs at the 3′-end and does not include coding sequences. The hypothesis by Keese and Gibbs needs to be confirmed by larger studies. Interestingly, in both studies the time of origin of the gene overlaps was estimated to take place after the divergence of mammals from birds.

As suggested by Miyata and Yasunaga (1978), the rate of evolution can be expected to be slower in overlapping genes. This is in agreement with a study by Lipman (1997) in which the higher rate of conservation of noncoding sequences of some genes is explained by the presence of antisense transcripts. However, there is not enough experimental evidence that higher conservation is a common feature of coding and noncoding overlapping genes. Shintani et al. (1999) found high 3′-UTR conservation in only one of two studied overlapping genes, and Svaren et al. (1997) found only one area with higher conservation in 3′-UTRs of overlapping *Stat6* and *Nab2* genes, and, even then, the authors expect this partial conservation to be due to some additional regulatory functions and not necessarily due to the overlap between the genes.

Here we report a study of 774 overlapping genes in human and 542 overlapping gene pairs in mouse as well as analysis of 778 human and mouse orthologous genes that, in at least one species, share exons with another gene.

RESULTS

Identification of Overlapping Genes

We used the NCBI human genome assembly Build 33 (April 2003) and the mouse genome assembly Build 30 (March 2003) as the sequence source for identification of overlapping genes. Out of 34,604 genes annotated in the human genome, we identified 774 pairs of overlapping genes, and of 33,936 analyzed genes in the mouse genome, we identified 578 pairs of overlapping genes.

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Table 1. Frequency of Different Types of Overlaps Between Protein-Coding Genes in Human and Mouse Genomes

	Human		Mouse	
	Overlapping genes	Genes with overlapping exons	Overlapping genes	Genes with overlapping exons
Total	774	542	578	455
Embedded	126 (16.28%)	15 (2.77%)	53 (9.17%)	7 (1.54%)
Tail to tail	414 (53.49%)	360 (66.42%)	314 (54.32%)	280 (61.54%)
Head to head	234 (30.23%)	167 (30.81%)	211 (36.51%)	168 (36.92%)
Involving coding sequence		299 (55.17%)		232 (50.99%)
Coding–coding overlap		57 (10.52%)		31 (96.81%)

We focused on annotated genomic sequence genes only and did not include ESTs to get high-quality data for mouse–human comparison. As shown by other studies (Wolfsberg and Landsman 1997) as well as our work in the early stages of this study, EST sequences can be identified as overlapping because of chimeric sequences, mislabeling, and genomic sequence contamination. More than 10% of such identified, overlapping genes are artifacts (Yelin et al. 2003). Because chimeric sequences can also be found among annotated mRNAs (Lehner et al. 2002), we used genomic localization to confirm the presence of gene overlaps in our study. Our earlier studies showed that simple presence of regions of complementarities between mRNAs is not sufficient and can lead to false-positive results. Because we wanted to compare human and mouse protein-coding genes, we excluded from our search noncoding genes, which in the genome scale are involved in ~75% of gene overlaps (Kiyosawa et al. 2003).

As shown in Table 1, among 774 overlapping protein coding genes in the human genome, 542 had overlapping exons. In 299 pairs of genes with overlapping exons, coding sequence was involved, and in 57 cases, coding sequences from both genes are coded by the same genomic fragment. From all human overlapping genes, 53% had tail-to-tail overlap (3′ to 3′), 30.23% showed head-to-head overlap (5′ to 5′), and 16.28% represented embedded genes. In the mouse genome, we found 578 pairs of protein-coding overlapping genes, and 455 of these pairs had overlapping exons. Of these, 232 pairs of genes with overlapping exons had coding sequence involved, and among these 31 pairs showed overlap between coding sequences of both genes. In mouse, 54.32% of genes overlapped at the 3′-ends, 36.51% of overlaps were head-to-head overlaps, and 9.17% of the gene pairs had one gene embedded into another. The fraction of gene pairs overlapping at the 5′-ends is significantly higher than previously reported by Shendure and Lehner, who found that only 5.53% (Shendure and Church 2002) and 15% (Lehner et al. 2002) of overlapping genes had head-to-head orientation. However, results similar to ours were presented by Yelin et al. (2003), who found that 31% of identified human overlapping genes overlap at the 5′-end. We also found 18 cases in the human genome and eight in mouse where one gene had exons overlapping with exons of not one but two different genes. These genes represent previously unreported triplets of overlapping genes. Table 2 lists all cases of such overlapping triplets. An example with three human overlapping genes—*MUTYH*, *TOE1*, and *TESK2*—is presented in Figure 1. The gene *TOE1* has overlapping exons with *MUTYH* at the 5′-end and with *TESK2* at the 3′-end. In the human genome we also found a segment with four exon overlapping genes: *LOC338549*, *IDI2*, *HT009*, and *IDI1*.

Identification of Human–Mouse Orthologs

We used NCBI HomoloGene data to identify mouse genes orthologous to overlapping human genes and vice versa. The 542 human gene pairs with overlapping exons could be divided into three categories—54 cases in which neither gene had a mouse homolog, 233 cases in which exactly one member of the gene pair had a mouse homolog, and 255 cases in which both members of the overlapping gene pair had mouse homologs annotated in HomoloGene. Among these 255 pairs with both mouse orthologs, in 95 cases genes were overlapping in both human and mouse, in 150 cases genes were overlapping in human but not in mouse although both mouse genes were mapped to the same contig, and in 10 cases identified homologs were in different contigs. The same search was performed using known mouse exon overlapping genes. For 455

Table 2. Human and Mouse Genes Participating in Multiple Overlaps

Human		
MUTYH	TOE1	TESK2
LOC348527	PRKCZ	LOC199990
MBDin	STAF65(gamma)	SLC4A1AP
AUP1	PRSS25	LOXL3
STAB1	FLJ12442	LOC285395
PKD2L2	C5orf5	LOC202047
HOXA3	LOC285943	HOXA4
LOC338549	IDI2	HT009
IDI2	HT009	IDI1
LOC283262	LOC347872	MGC5306
HSPC134	LOC338899	LOC145553
FLJ20190	LOC145694	KIF23
SNN	LOC51061	FLJ21777
LOC339053	MGC35048	LOC283827
DRG2	LOC147229	MYO15A
AKAP8	LOC284430	NAKAP95
MCOLN1	LOC147791	NTE
LOC286522	RPL10	DNASE1L1
Mouse		
AW105885	2010003O02Rik	LOC230075
5830400N10Rik	LOC329840	AI563590
Pex1	C030048B08Rik	1700109H08Rik
Lass1	Rent1	B430202H16Rik
Ilf3	LOC330902	Tgut-pending
Zfp278	LOC327858	LOC216505
1110031I02Rik	LOC238023	MGC28978
Vdac2	1810030M08Rik	A430057M04Rik
LOC277250	5830462I21Rik	MGC28827

Genes in the middle column have exons overlapping with exons of other genes at both 3′- and 5′-ends.

Figure 1 Overlap between three human genes: *MUTH*, *FLJ13949*, and *TESK2*. Dark boxes represent coding sequence. Light boxes represent untranslated regions.

pairs of these genes, in 36 cases neither of the two overlapping genes had an annotated human homolog, in 179 cases only one gene had a human homolog, and in 240 cases both homologs were found. Out of 240 cases in which both human homologs were identified, in 95 instances genes overlapped in both human and mouse (as expected from the above results), in 144 cases both human homologs were in the same contig but did not overlap, and in one case human homologs were on different contigs.

From the entire set of homologous genes, for further analysis, we selected only those where both homologs were found and both were on the same contig. The presence of the overlap in both organisms was not required. This gave us 389 pairs (778 genes) of human and mouse orthologous overlapping genes in which in at least one species, overlapping genes shared not only genomic location but also exonic sequence.

Human–Mouse Sequence Conservation

It was observed that a large fraction of the mammalian genes show high conservation of 5′- and 3′-untranslated regions. Lipman (1997) suggested that a subset of these cases can be explained by the existence of overlapping antisense transcripts. We analyzed the set of human and mouse orthologous genes overlapping in at least one of the organisms. We excluded any pair for which only one homolog was identified as well as all cases in which identified homologs were in different locations to eliminate possible cases of paralogous and not orthologous sequence assignment. After aligning all putative orthologous sequences, we additionally eliminated from our set all genes that had a similarity level between human and mouse coding sequences below 69%. Such low similarity between sequences may indicate that we may not have a true ortholog, and therefore we chose not to include them. The cutoff threshold of 69% was chosen based on the distribution of human–mouse ortholog similarities (W. Makalowski, unpubl.). We found 44 such cases, and these orthologs as well as their overlapping counterparts were eliminated from the set. We excluded all orthologous pairs when more than one homolog was annotated in HomoloGene. This gave us a set of 638 human–mouse orthologs overlapping with another gene in at least one organism.

We compared untranslated regions and coding sequences of the human and mouse orthologs. At first we analyzed the entire set of 638 orthologs, regardless of category of the overlap. We compared separately all 5′-UTRs, coding sequences, and 3′-UTRs. From our comparison we discarded all alignments that were shorter than 30 bp. The median value of the CDS identity between human and mouse genes was 85.67%, for 3′-UTRs 63.89%, and for 5′-UTRs 61.54%. These values do not differ significantly when compared with the reported results for all known human–mouse orthologs (Makalowski and Boguski 1998) because all mean values lie within one standard deviation (Table 3). Although overlapping genes in our data set overall did not demonstrate greater than average conservation, we expected a higher level of similarity when coding regions were involved. We, therefore, partitioned our sequences based on the type of overlap and calculated identity statistics for each set. Our main focus was on the comparison of coding sequence when coding sequence from both genes was involved in the overlap, comparison of 5′-UTRs when 5′-UTRs were overlapping with the coding sequence of another gene, and 3′-UTRs when they were overlapping with the coding region of another gene. We analyzed these values when the above overlap was observed in at least one organism as well as when the particular type of overlap was observed in both organisms. In neither case did we observe significantly higher conservation of coding sequence or untranslated regions. Table 3 presents the results of our analysis. One can see that mean and median values do not differ significantly either between categories or when compared with overall human–mouse sequence identities. In all cases, the mean and median values obtained in our study lie within the range of one standard deviation reported for all human–mouse orthologs by Makalowski and Boguski (1998).

Table 3. Summary of Sequence Similarities Between Untranslated Regions and Coding Sequences of Human and Mouse Orthologous Overlapping Genes

	5′-UTR				CDS				3′-UTR			
Overlap	**no. of seq.**	**median**	**mean**	**std**	**no. of seq.**	**median**	**mean**	**std**	**no. of seq.**	**median**	**mean**	**std**
Sequences that have overlaps in at least one of the species												
Coding–coding	15	66.67	67.11	12.46	34	**84.48**	**84.9**	4.54	30	61.03	60.7	15.98
3UTR_Coding	102	60.96	61.75	15.09	185	85.36	84.66	4.95	165	**63.23**	**63.35**	13.52
5UTR_Coding	38	**60.72**	**61.92**	15.42	67	84.34	84	4.63	60	59.49	62.57	13.04
5UTR_5UTR	61	63.53	63.06	13.74	91	84.75	84.46	5.11	81	61.27	62.74	12.11
3UTR_3UTR	281	61.01	61.67	14.45	483	85.95	85.12	5.17	440	64.83	64.68	13.07
Sequences that have overlaps in both of the species												
Coding–coding	0	0	0	N/A	0	0	0	N/A	0	0	0	N/A
3UTR_Coding	20	55.76	58.08	15.92	34	84.9	84.2	4.51	33	63.41	62.5	12.11
5UTR_Coding	1	72.7	72.7	N/A	2	88.97	88.97	2.89	2	69.48	69.48	5.79
5UTR_5UTR	3	72.52	69.53	5.33	4	86.71	85.3	6.04	4	69.48	69.94	10.97
3UTR_3UTR	74	60.7	61.26	15.41	124	85.34	85.02	4.8	120	69.06	68.27	11.47
Large scale human–mouse comparison (Makalowski and Boguski 1998)												
	871	67	69.7	12.9	1,138	89	86.4	12.3	938	69.4	71	12.2

In bold are values that we expected to be significantly higher because of the overlap with another gene coding sequence and therefore be under higher selection against mutations.

Conservation of Gene Structure and Overlap Pattern

Besides sequence similarity, we also studied the conservation of the gene structure and overlap pattern. As mentioned above, only in 95 out of 255 cases were mouse homologs of overlapping human genes involved in overlaps in the mouse genome. A significant fraction of these 95 pairs show different overlap patterns in the two genomes. This is in contrast to the data presented by Shendure and Church (2002), where in all cases of human and mouse orthologous overlapping genes, the pattern of overlap was the same in both organisms. In many cases, lack of similarity could be explained by unfinished sequencing of one or both UTRs. For example, in the human gene *SRR*, the 3′-UTR sequence overlaps with the 3′-UTR and coding region of *FLJ10534*. In mouse, the 3′-UTR of *Srr* does not overlap with a coding region of the gene *LOC193029*, but only overlaps with the 3′-UTR. However, the 3′-UTR of *LOC193029* overlaps with both a coding region and the 3′-UTR of *Srr*. Although there is a reversed pattern of overlaps, it is apparent that the 3′-UTRs of human *FLJ10534* and mouse *Srr* are very short, and, if extended, they would overlap with coding sequences of the gene sharing the same locus. Based on this observation, we can expect that the 3′-UTRs of both genes should overlap with both the 3′-UTR and coding sequence of another gene if they were fully sequenced. Figure 2 shows a few examples of genes in which the overlap pattern is different in human and mouse and cannot be explained by the missing ends of 3′- or 5′-UTRs. One explanation of the above discrepancies in genome structure and overlap pattern could be that we are observing different splice variants of one or both genes in each organism. For example, Figure 3A shows that the 5′-UTR of *SPA17* overlaps with the 5′-UTR of one *CSE-C* splice variant (variant b), and that the second exon of *SPA17*, containing coding sequence, overlaps with the third noncoding exon of *CSE-C* variant a. Therefore, depending on which splice variant we look at, we see a different overlap pattern. In mouse, only one splice variant, with a missing 5′-UTR, is annotated on the map, and there is no overlap between the orthologous genes. Problems with different overlap patterns could also be caused by incorrect annotation. An example of such a case is the *ASE-1* gene (Fig. 3B). In human, the 3′-UTR of *ASE-1* overlaps with the 3′-UTR and coding sequence of *ERCC1*. In mouse, *2610103M17Rik*, the ortholog of *ASE-1*, does not overlap with *Ercc1*. However, there is another gene placed on the mouse genome map between *Ercc1* and *2610103M17Rik*, *LOC330481*. This gene overlaps at the 3′-end with *Ercc1* and at the 5′-end with *2610103M17Rik*. It seems likely that *LOC330481* is a part of the *2610103M17Rik* 3′-UTR and not a separate gene, and therefore both are parts of the mouse *ASE-1* ortholog. If this is true, the pattern of overlaps in human and mouse does not differ in this case.

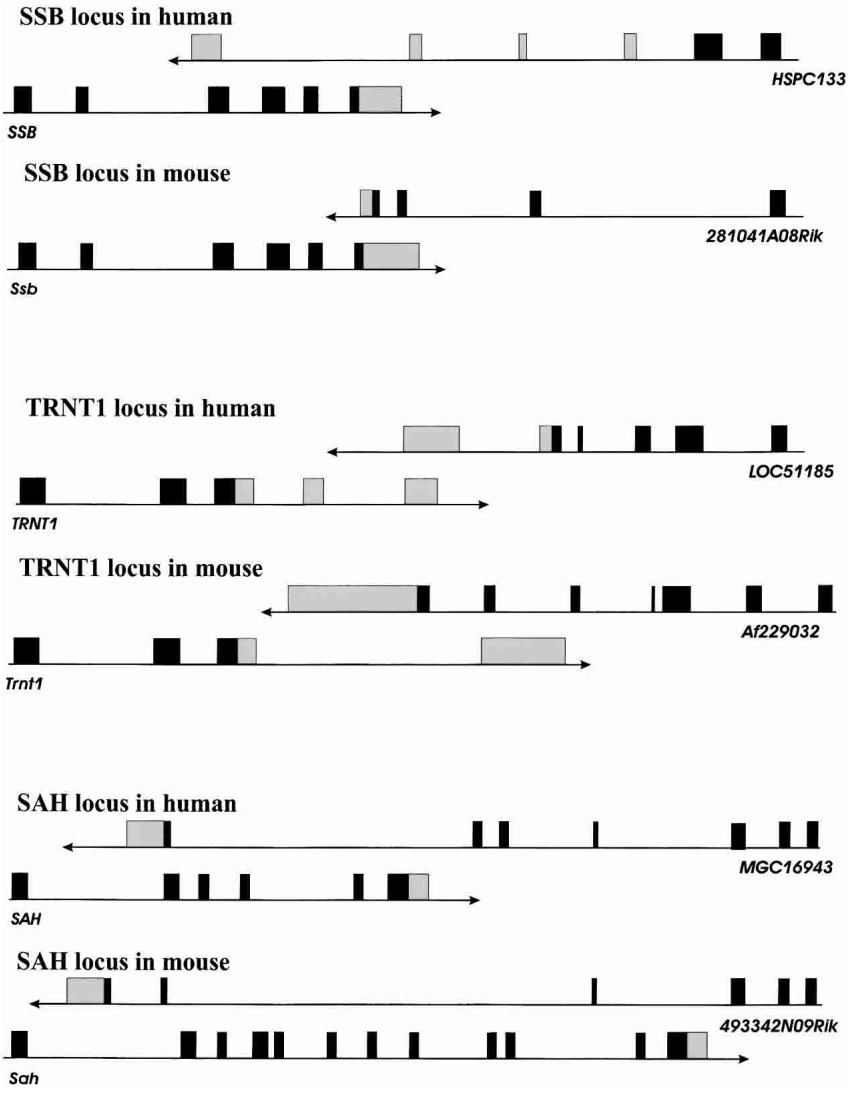


Figure 2 Examples of genes with different patterns of overlap in human and mouse. Dark boxes represent coding sequence. Light boxes represent untranslated regions.

DISCUSSION

We identified >774 gene pairs sharing a locus in the human genome and 542 in the mouse genome. It was expected that overlapping genes, especially those in which coding sequences are involved, would be more conserved between species than nonoverlapping genes (Lipman 1997; Yelin et al. 2003). This is mostly because a mutation in the overlapping region would cause changes in both genes, and the selection against these mutations should therefore be stronger. This hypothesis does not hold in our data. The identity between overlapping human and mouse orthologs was not higher than average. Even when we limited the comparison to genes in which a particular overlap was observed in both species, we did not notice any significant difference in conservation of coding sequences or untranslated regions. We also noticed that the pattern of overlap can be different across species. The lack of higher conservation rates and differing overlap patterns raises interesting questions about the evolution of overlapping genes. The hypothesis of overlapping genes origin suggested by Shintani et al. (1999) cannot fully explain these phenomena because it is related to 3′-end overlaps only and does not consider 5′-overlaps at all. Overprinting as the origin of overlapping genes, as suggested by Keese and Gibbs (1992), may provide a better explanation,

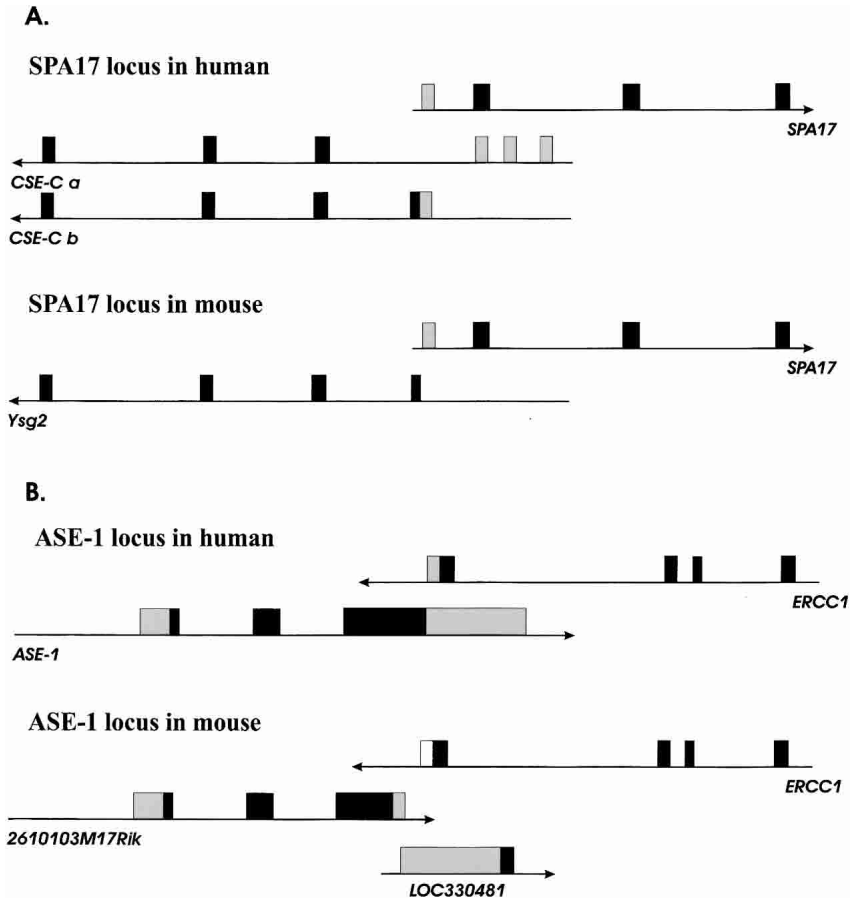


Figure 3 Examples of different patterns of overlap in human and mouse caused by different splice variants or annotation error. Dark boxes represent coding sequence. Light boxes represent untranslated regions.

but more detailed studies would be required. However, because overprinting generates novel genes, the hypothesis may help in deciphering some observations. Keese and Gibbs suggest that overprinting took place after the divergence of mammals from birds, and that overlapping genes represent young, phylogenetically restricted genes encoding proteins with diverse functions, and are therefore specialized to the present life-style of the organism in which they are found. This could explain why we could not find orthologs of human overlapping genes in *Caenorhabditis elegans*. The lineage-specific nature of the overlapping genes, as suggested by Shendure and Church, may explain only partial intersection between the human and mouse gene sets. We observed the lack of many mouse orthologs of human overlapping genes and many cases in which only one ortholog was found. Although we suspect that sometimes these orthologs have simply not been identified yet, this may not explain cases in which one ortholog was found but none of the neighboring, closely localized genes matched the other gene. Of course, at this point we cannot exclude the possibility that in cases like the above we have paralogs and not orthologs. Additionally, the overlap is not always repeated in both human and mouse, and the structure of genes involved in the overlap sometimes differs as well. This could mean that overlaps can be generated by multiple mechanisms, including overprinting, as suggested by Keese and Gibbs, or rearrangements or loss of parts of 3′-UTRs and use of neighboring gene signals, as suggested by Shintani et al. In our data set, we can find cases supporting each of these mechanisms, as well as implying that overlaps between some genes could be specific to mammals, and others could have happened after separation of mouse and human. This observation is made based on the fact that, in some cases, the lack of an overlap in one of the species cannot be explained by unfinished sequencing, alternative splice variants, or wrong annotations, as well as the fact that homologs of overlapping genes were sometimes found on different contigs or even different chromosomes. We excluded all such cases from our human–mouse comparison because of the possibility of paralogy. In many instances, those were the only homologous sequences identified. If these are orthologous genes, in addition to rearrangement, some other mechanism would be required to explain divergence, such as duplication of the overlapping gene region followed by loss of one gene from each duplicate. However, further and more detailed studies including more lineages are required to confirm any of the above hypotheses.

METHODS

Sequence Data

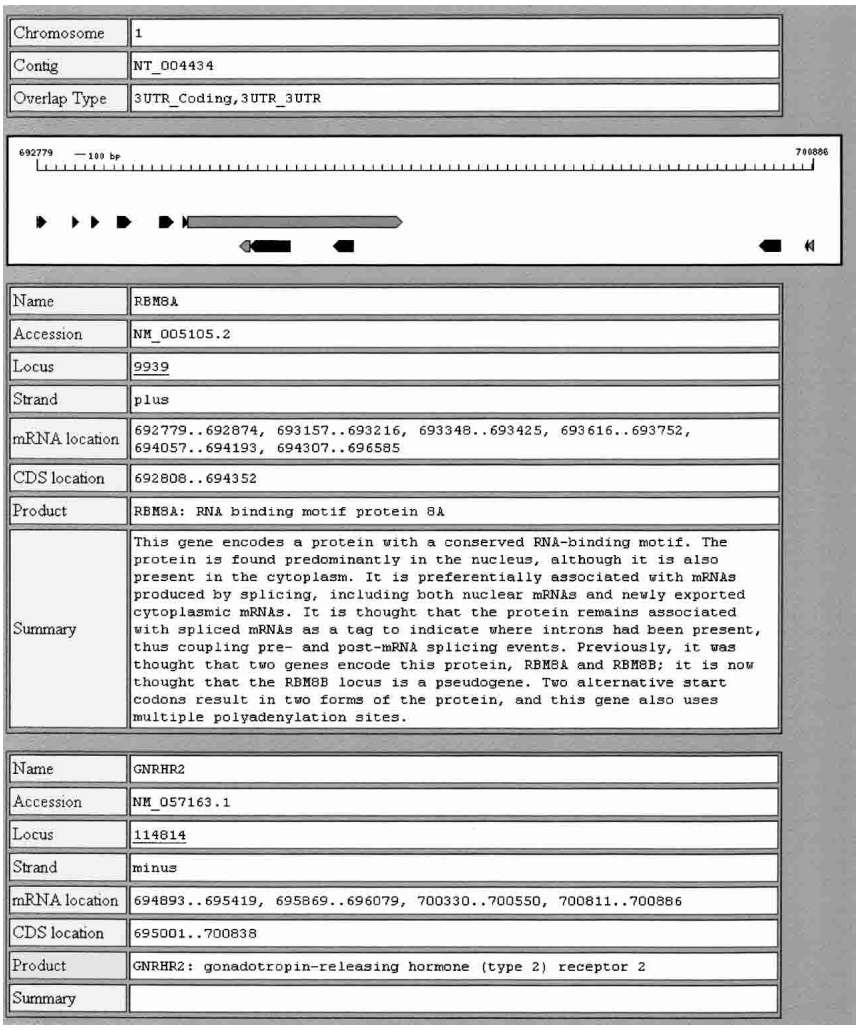
We analyzed assembled and annotated human and mouse genomic sequences from GenBank to identify overlapping genes. We used NCBI's Build 33 release of the human genome (April 2003) and Build 30 of the mouse genome (March 2003).

Identification of the Overlapping Genes

The GenBank files contain annotations for both gene and mRNA features. However, the location of the gene tag is not very well defined and is not consistent. The gene feature location may or may not include the promoter region and regulatory elements. Therefore, in the interest of consistency, we defined the gene locations in terms of the mRNA location, which is represented by the set of intervals on the genomic region, referring to the particular mRNA exons.

Identification of Human/Mouse Orthologs and Their Authentication

Mouse orthologs of human overlapping genes were identified by searching the HomoloGene database at NCBI. We searched the HomoloGene database for mouse orthologs of every human overlapping gene we identified, and vice versa. All cases in which no ortholog was identified for either of the two overlapping genes were excluded from further search. We also excluded all cases in which genes were not located on the same contig to avoid the analysis of paralogous and not orthologous genes as well as genes in which more than one homolog was annotated in the HomoloGene database. Furthermore, all orthologous pairs that had coding sequence identity below 69% were eliminated to avoid the possibility of analysis of paralogs. The 69% cutoff level was chosen based on the distribution of coding sequences identities of human and mouse orthologs (W. Makalowski, unpubl.). We also excluded overlapping counterparts of genes with low identity to be consistent with the requirement of including only those genes for which both orthologs, for a given overlapping gene pair, were identified.



Chromosome	1
Contig	NT_004434
Overlap Type	3UTR_Coding,3UTR_3UTR

692779 — 100 bp — 700886

Name	RBM8A
Accession	NM_005105.2
Locus	9939
Strand	plus
mRNA location	692779..692874, 693157..693216, 693348..693425, 693616..693752, 694057..694193, 694307..696585
CDS location	692808..694352
Product	RBM8A: RNA binding motif protein 8A
Summary	This gene encodes a protein with a conserved RNA-binding motif. The protein is found predominantly in the nucleus, although it is also present in the cytoplasm. It is preferentially associated with mRNAs produced by splicing, including both nuclear mRNAs and newly exported cytoplasmic mRNAs. It is thought that the protein remains associated with spliced mRNAs as a tag to indicate where introns had been present, thus coupling pre- and post-mRNA splicing events. Previously, it was thought that two genes encode this protein, RBM8A and RBM8B; it is now thought that the RBM8B locus is a pseudogene. Two alternative start codons result in two forms of the protein, and this gene also uses multiple polyadenylation sites.

Name	GNRHR2
Accession	NM_057163.1
Locus	114814
Strand	minus
mRNA location	694893..695419, 695869..696079, 700330..700550, 700811..700886
CDS location	695001..700838
Product	GNRHR2: gonadotropin-releasing hormone (type 2) receptor 2
Summary	

Figure 4 The screenshot from the overlapping genes database presenting overlap details.

Human–Mouse Comparison

We downloaded mRNA sequences of all orthologous human and mouse genes. We used GenBank annotations to separate coding sequence and untranslated regions for similarity search. All sequences were aligned using map software (Huang 1994) with the following parameters: a mismatch penalty, −3; match, 10; gap opening penalty, 50; gap extension penalty, 5; longest penalized gap, 10. Then, identities (excluding gaps) between coding sequences, 5′-UTRs, and 3′-UTRs were calculated. We discarded from our comparison all alignments shorter than 30 bp.

Overlapping Genes Database

All overlap information that could be inferred from the annotated GenBank files for Build 33 of the human genome sequence and Build 30 of the mouse genome sequence is stored in a MySQL database. A Web-based graphical user interface to the data is available at http://posnania.cbio.psu.edu/research/overlapping_genes.html. The interface allows users to search for known overlapping genes by name, accession number, or LocusLink id. A second form allows users to browse the set of overlaps by overlap type (Coding–Coding, 3′-UTR–Coding, etc). Overlaps are displayed in a pictorial format with colors being used to distinguish untranslated regions from coding regions. Information retrieved dynamically from LocusLink is used to summarize the genes participating in the overlaps (see example in Fig. 4).

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REFERENCES

Bachman, N.J., Wu, W., Schmidt, T.R., Grossman, L.I., and Lomax, M.I. 1999. The 5′ region of the COX4 gene contains a novel overlapping gene, NOC4. *Mamm. Genome* **10:** 506–512.

Burke, J., Wang, H., Hide, W., and Davison, D.B. 1998. Alternative gene form discovery and candidate gene selection from gene indexing projects. *Genome Res.* **8:** 276–290.

Cooper, P.R., Smilinich, N.J., Day, C.D., Nowak, N.J., Reid, L.H., Pearsall, R.S., Reece, M., Prawitt, D., Landers, J., Housman, D.E., et al. 1998. Divergently transcribed overlapping genes expressed in liver and kidney and located in the 11p15.5 imprinted domain. *Genomics* **49:** 38–51.

Huang, X. 1994. On global sequence alignment. *Comput. Appl. Biosci.* **10:** 227–235.

Keese, P.K. and Gibbs, A. 1992. Origins of genes: "Big bang" or continuous creation? *Proc. Natl. Acad. Sci.* **89:** 9489–9493.

Kiyosawa, H. and Abe, K. 2002. Speculations on the role of natural antisense transcripts in mammalian X chromosome evolution. *Cytogenet. Genome Res.* **99:** 151–156.

Kiyosawa, H., Yamanaka, I., Osato, N., Kondo, S., and Hayashizaki, Y. 2003. Antisense transcripts with FANTOM2 clone set and their implications for gene regulation. *Genome Res.* **13:** 1324–1334.

Lazar, M.A., Hodin, R.A., Darling, D.S., and Chin, W.W. 1989. A novel member of the thyroid/steroid hormone receptor family is encoded by the opposite strand of the rat c-erbA α transcriptional unit. *Mol. Cell. Biol.* **9:** 1128–1136.

Lehner, B., Williams, G., Campbell, R.D., and Sanderson, C.M. 2002. Antisense transcripts in the human genome. *Trends Genet.* **18:** 63–65.

Lipman, D.J. 1997. Making (anti)sense of non-coding sequence conservation. *Nucleic Acids Res.* **25:** 3580–3583.

Makalowski, W. and Boguski, M.S. 1998. Evolutionary parameters of the transcribed mammalian genome: An analysis of 2,820 orthologous rodent and human sequences. *Proc. Natl. Acad. Sci.* **95:** 9407–9412.

Misener, S.R. and Walker, V.K. 2000. Extraordinarily high density of unrelated genes showing overlapping and intraintronic transcription units. *Biochim. Biophys. Acta* **1492:** 269–270.

Miyajima, N., Horiuchi, R., Shibuya, Y., Fukushige, S., Matsubara, K., Toyoshima, K., and Yamamoto, T. 1989. Two erbA homologs encoding proteins with different T3 binding capacities are transcribed from opposite DNA strands of the same genetic locus. *Cell* **57:** 31–39.

Miyata, T. and Yasunaga, T. 1978. Evolution of overlapping genes. *Nature* **272:** 532–535.

Morelli, C., Magnanini, C., Mungall, A.J., Negrini, M., and Barbanti-Brodano, G. 2000. Cloning and characterization of two overlapping genes in a subregion at 6q21 involved in replicative senescence and schizophrenia. *Gene* **252:** 217–225.

Normark, S., Bergstrom, S., Edlund, T., Grundstrom, T., Jaurin, B., Lindberg, F.P., and Olsson, O. 1983. Overlapping genes. *Annu. Rev. Genet.* **17:** 499–525.

Okazaki, Y., Furuno, M., Kasukawa, T., Adachi, J., Bono, H., Kondo, S., Nikaido, I., Osato, N., Saito, R., Suzuki, H., et al. 2002. Analysis of

the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. *Nature* **420:** 563–573.
Shendure, J. and Church, G.M. 2002. Computational discovery of sense–antisense transcription in the human and mouse genomes. *Genome Biol*. **3:** RESEARCH0044.
Shintani, S., O'hUigin, C., Toyosawa, S., Michalova, V., and Klein, J. 1999. Origin of gene overlap: The case of TCP1 and ACAT2. *Genetics* **152:** 743–754.
Slavov, D., Hattori, M., Sakaki, Y., Rosenthal, A., Shimizu, N., Minoshima, S., Kudoh, J., Yaspo, M.L., Ramser, J., Reinhardt, R., et al. 2000. Criteria for gene identification and features of genome organization: Analysis of 6.5 Mb of DNA sequence from human chromosome 21. *Gene* **247:** 215–232.
Svaren, J., Apel, E.D., Simburger, K.S., Jenkins, N.A., Gilbert, D.J., Copeland, N.A., and Milbrandt, J. 1997. The Nab2 and Stat6 genes share a common transcription termination region. *Genomics* **41:** 33–39.
Williams, T. and Fried, M. 1986. A mouse locus at which transcription from both DNA strands produces mRNAs complementary at their 3′ ends. *Nature* **322:** 275–279.
Wolfsberg, T.G. and Landsman, D. 1997. A comparison of expressed sequence tags (ESTs) to human genomic sequences. *Nucleic Acids Res*. **25:** 1626–1632.
Yelin, R., Dahary, D., Sorek, R., Levanon, E.Y., Goldstein, O., Shoshan, A., Diber, A., Biton, S., Tamir, Y., Khosravi, R., et al. 2003. Widespread occurrence of antisense transcription in the human genome. *Nat. Biotechnol*. **21:** 379–386.
Zhuo, D., Zhao, W.D., Wright, F.A., Yang, H.Y., Wang, J.P., Sears, R., Baer, T., Kwon, D.H., Gordon, D., Gibbs, S., et al. 2001. Assembly, annotation, and integration of UNIGENE clusters into the human genome draft. *Genome Res*. **11:** 904–918.

WEB SITE REFERENCES

http://posnania.cbio.psu.edu/research/overlapping_genes.html; Overlapping Genes Project.

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