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The Haemochromatosis Candidate Gene HFE (HLA-H) of Man and Mouse Is Located in Syntenic Regions Within the Histone Gene Cluster

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Abstract The HFE (HLA-H) gene is a strong candidate gene for hereditary haemochromatosis and was localized on the short arm of chromosome 6 to 6p21.3-p22. In addition, the sequence of the homologous mouse and rat cDNA and a partial sequence from the mouse gene have been reported recently. In this report, we describe the location of the human and the mouse HFE (HLA-H) gene within the histone gene clusters on the human chromosome 6 and the mouse chromosome 13. Both the human and the murine gene were located on syntenic regions within the histone gene clusters in the vicinity of the histone H1t gene. The genomic sequence of the human HFE (HLA-H) gene and the 3′ portion of the homologous mouse gene were determined. Comparison of the genomic sequences from man and mouse and the cDNA sequence from rat shows significant similarities, also beyond the transcribed region of the mouse gene. J. Cell. Biochem. 69:117–126, 1998.

Key words: haemochromatosis gene; histone gene cluster; YACs; cosmid contig; sequences; species comparison

Histones are small basic proteins responsible for the formation of chromatin structure and involved in transcriptional regulation [Wolffe, 1995]. The family of histone proteins may be subdivided into the group of core histones (H2A, H2B, H3, and H4), which form the nucleosomal core structure, and the H1 linker histones, which are necessary for the formation of higher order chromatin structures [Thoma et al., 1979]. Each class of histones consists of several subtypes, which are encoded by nonallelic gene variants. In many organisms, core and linker histone genes form clusters containing several histone genes. These clusters may be arranged as tandem repeats of genes coding for the five histone classes, such as in the genomes of *Drosophila melanogaster* or of sea urchins, where several hundreds of histone gene quintets are arranged in tandem [Maxson et al., 1983]. In contrast, analysis of histone genes in the chicken, murine, and human genomes has shown that histone genes are clustered, but not tandemly repeated in the genomes of these organisms [Heintz et al., 1981]. Previously, we have isolated the complete set of human H1 histone genes [Doenecke and Toenjes, 1986; Eick et al., 1989; Albig et al., 1991, 1997a] and located all of these genes (except H1°) and the neighboring core histone genes to 6p21.3-p22 [Albig et al., 1993; Doenecke et al., 1994], and all five murine replication-dependent H1 histone genes and the H1t gene on mouse chromosome 13 [Drabent et al., 1995a], within a region corresponding to 6p21.3. Using radiation hybrids, the human H1 histone genes were localized about 6.5 cM telomeric of HLA-A [Volz et al., 1994]. Recently, we have presented the arrangement and the sequences of 35 histone genes within the major human histone gene cluster on the short arm of chromosome 6 [Albig et al., 1997b]. Similarly, the arrangement of mouse histone genes within a major histone gene cluster on the corresponding region of mouse chromosome 13 was recently described by Wang et al. [1996]. Feder et al. [1996] have localized the HFE (HLA-H) gene, a MHC class I related gene and a strong candidate gene for

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haemochromatosis, also to the region 6p21.3-p22.

Hereditary haemochromatosis is an autosomal recessive disorder of iron metabolism. It represents one of the most common inherited diseases among individuals of Northern European descent [Cox, 1996]. Linkage studies have demonstrated that the haemochromatosis gene is located on the short arm of chromosome 6 in close association with the HLA-A gene locus [Simon et al., 1976]. Further studies had indicated linkage with the markers D6S105 [Jazwinska et al., 1993] and D6S1260 [Raha-Chowdhury et al., 1995], but intensive studies of many groups within the past two decades failed to identify the gene for this disorder. Factors contributing to these difficulties were insufficient clone coverage, and lack of appropriate markers and families to observe sufficient recombinants in hereditary haemochromatosis. In particular, linkage disequilibrium was observed within a large region when long distances were studied between markers with similar disequilibrium to hereditary haemochromatosis. Feder et al. [1996] have identified the haemochromatosis locus by analysis of the whole haemochromatosis candidate region. Their studies were based on a linkage disequilibrium mapping approach combined with maximum allelic association and identification of potential recombination events that may have occurred in the past. We have localized within the major histone gene cluster the markers described by Feder et al. [1996] surrounding the haemochromatosis gene [Albig et al., 1997b]. We therefore concluded that the HFE (HLA-H) gene must be localized in the vicinity of the histone gene cluster or within this cluster, which covers 260 kb within the haemochromatosis gene region at 6p21.3-p22 [Albig et al., 1993, 1997b].

In order to identify the precise localization of the HFE (HLA-H) gene within the histone gene cluster and to determine the gene structure and the genomic sequence of the HFE (HLA-H) gene, we analyzed the cosmid contig of the major histone gene cluster, which we had established upon analysis of this cluster [Albig et al., 1997b]. In parallel studies, we had isolated in recent years several portions of the murine histone gene cluster and could show that it maps to a region (13A2.3) that corresponds to the human histone gene cluster region [Drabent et al., 1995a]. Thus, we were able to compare the genomic organization of the murine and human HFE (HLA-H) genes and their relation to the histone gene cluster.

MATERIALS AND METHODS

Screening the Cosmid Contig With PCR

Cosmids from the contig of the major histone gene cluster [Albig et al., 1997b] and phage clones bearing the H1t gene [Drabent et al., 1995b] were screened by PCR. Primers were deduced from the published sequences of the human and murine cDNAs [Acc. no.: U60319, Feder et al., 1996; Acc. no.: U66849, Hashimoto et al., 1997]. PCR analyses were performed in a 100-µl reaction volume in a buffer containing 300 pmol of oligonucleotide primer, 10 ng of cosmid or phage DNA, 200 µM of each of the four dNTPs, and 2.5 U of Taq DNA polymerase (AGS, Heidelberg, Germany). The conditions of amplification included a first denaturation at 94°C, annealing at 55°C, and amplification at 72°C. The positive cosmids and phage were identified by detecting the appropriate amplificate in agarose gel electrophoresis.

Mapping and Subcloning of the Cosmid and Phage Insert DNA

DNA from appropriate clones was prepared by standard techniques [Sambrook et al., 1989]. DNA was cut with *Eco*RI and the resulting fragments were analyzed by Southern blotting [Southern, 1975] and subsequent hybridization with labeled PCR amplificates as probes. Probes from the 5′ and the 3′ end of the HFE (HLA-H) gene were generated by PCR. The 5′ probe was a 118-bp fragment amplified with the primer pair Oligo 1 (TCACAAGCAGGTACCTCTTG) and Oligo 2 (ACCCTACAGCTATTGCCTAG). The 3′ probe was a 104-bp fragment amplified with the primer pair Oligo 3 (TGTGATTTACGCTCATTGTA) and Oligo 4 (CTAACAGGTAATAGACAAGT). The probe from the 3′ portion of the mouse HFE (HLA-H) homologue was amplified with the primer pair Oligo 5 (AACCTCTCCCTCCAGAACTG) and Oligo 6 (CCCCGGATGAAGGTTCACAAC). The PCR amplification yielded a 168-bp fragment. The DNA fragments were isolated from 2% agarose gels with the DNA extraction kit (QIAEX) from Qiagen (Hilden, Germany) and labeled with the Rediprime random prime kit from Amersham (Braunschweig, Germany). The cosmid and phage fragments, which gave a hybridization signal, and the fragments between these end

fragments deduced from the map of these cosmids (Albig et al., 1997b) were subcloned in pUC19. The subcloned insert fragments were further mapped by combinatorial restriction endonuclease cleavage.

Sequencing and Sequence Analysis

The subcloned fragments were directly sequenced by the dideoxy method within double-stranded plasmid DNA at the reverse and universal primer binding sites. The transitions of the *Eco*RI fragments were confirmed by sequencing with sequence specific primers deduced from the sequencing results of the *Eco*RI-generated fragments. The ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Norwalk, CT) was used for the sequencing reaction. Sequence analysis was done on an ABI 373A DNA Sequencer (Applied Biosystems, Foster City, CA). Binding sites for transcription factors were searched with the TFsearch program. Homology searches were done with the MegAlign program of the DNAstar (Madison, WI) software.

RESULTS

Recently, we have shown that the chromosomal marker D6S2239, which is associated with the candidate gene responsible for hereditary haemochromatosis [Feder et al., 1996], is located within the major human histone gene cluster on the short arm of chromosome 6 in the region 6p21.3-p22 [Albig et al., 1997b]. For the localization of the HFE (HLA-H) gene within this histone gene cluster, we screened the cosmid contig of the histone gene cluster region by PCR amplification with primer pairs deduced from the sequence of the 5′ and the 3′ ends of the human HFE (HLA-H) cDNA [Feder et al., 1996]. In this screen, only one cosmid clone (6E8) was positive with both primer pairs. Two other cosmids (7A7 and 6H3) were positive with the primer pair from the 5′ end, whereas cosmid 7G4 was positive with the primer pair derived from the 3′ end. For mapping the 5′ and 3′ ends of the HFE (HLA-H) gene within the positive cosmids, we used the radioactively labeled PCR-products as probes for hybridization of the Southern blot with *Eco*RI digested DNA of the positive cosmids. A 3.5-kb fragment of the cosmids 7A7, 6H3, and 6E8 was positive with the 5′ specific probe and a 9-kb fragment from cosmids 6E8 and 7G4 was positive with the 3′ specific probe. Thus, we were able to assign the hybridizing fragments within the cosmid map of the histone gene cluster and found the gene localization as documented in Figure 1. The HFE (HLA-H) gene spans a region of at least 10 kb. The transcribed region of the gene ends about 7 kb upstream of the H4/g gene, which is located in the vicinity of the H1t gene [Drabent et al., 1993], and the transcriptional orientation is opposite to that of the H4/g gene.

The association of a histone H4 gene with the H1t gene is conserved in man, mouse, and rat [Drabent et al., 1995b]. Since the human HFE (HLA-H) gene maps in the vicinity of this gene pair, we have analyzed a phage clone (G1.1) containing the mouse H1t and H4 genes from chromosome 13 for the presence of the mouse haemochromatosis gene by PCR-amplification. On the basis of the sequence information obtained from the murine HFE (MR2) homologous cDNA deposited in the EMBL nucleotide sequence database [Hashimoto et al., 1997], we synthesized a primer pair homologous to the 3′ end of this cDNA. The primers were used for PCR amplification with DNA from phage G1.1. The amplified fragment was analyzed by automatic sequencing with the dye-terminator technique. DNA from lambda phage G1.1, isolated from a mouse genomic library from the mouse strain 129, was digested with several restriction endonucleases and analyzed for the presence of HFE (MR2) sequences within the separated fragments by Southern blot. The DNA fragments were transferred to nylon membrane and were hybridized with the labelled PCR fragment. We found hybridization within a 3-kb *Eco*RI fragment at a distance of about 4 kb from the histone H4 gene in the mouse genome.

For further comparison, we have sequenced the entire human HFE (HLA-H) gene and the 3′ portion of the mouse HFE (MR2) gene. The sequences of the human and murine HFE genes have been deposited with the EMBL nucleotide sequence data library with the accession numbers Z92910 and Y12650, respectively. The human sequence data were recently confirmed by the analysis of Ruddy et al. [1997] who have analysed a YAC clone in detail, which overlaps the YAC clone we have used for our studies of the human histone gene cluster. From our sequence data and the sequences of the cDNAs we deduced the gene structures shown in Figure 2. The mRNA of the human gene is generated by 7 exons of varied size. Comparison of

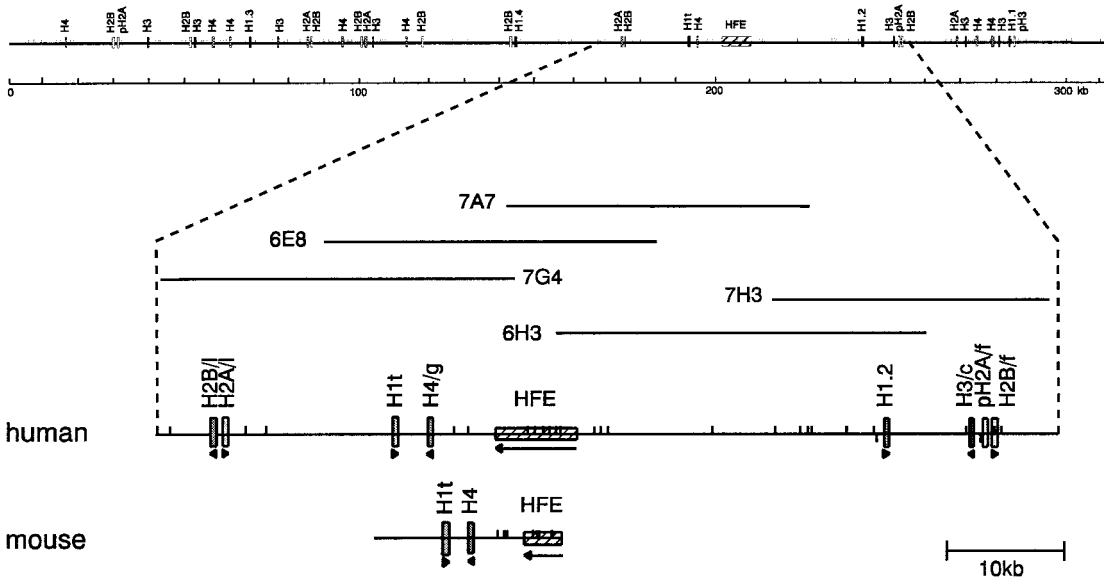

Fig. 1. **Top:** Organization of the human major histone gene cluster. Histone genes are indicated as vertical rectangles. Restriction sites (vertical lines): *Eco*RI, above; *Mlu*I, below. **Bottom:** Detailed organization of the HFE gene within the histone gene cluster in the human and the murine genome. Cosmids from the histone gene cluster contig [Albig et al., 1997b] are indicated. Orientations of the histone ORFs are indicated as arrowheads below the rectangles.

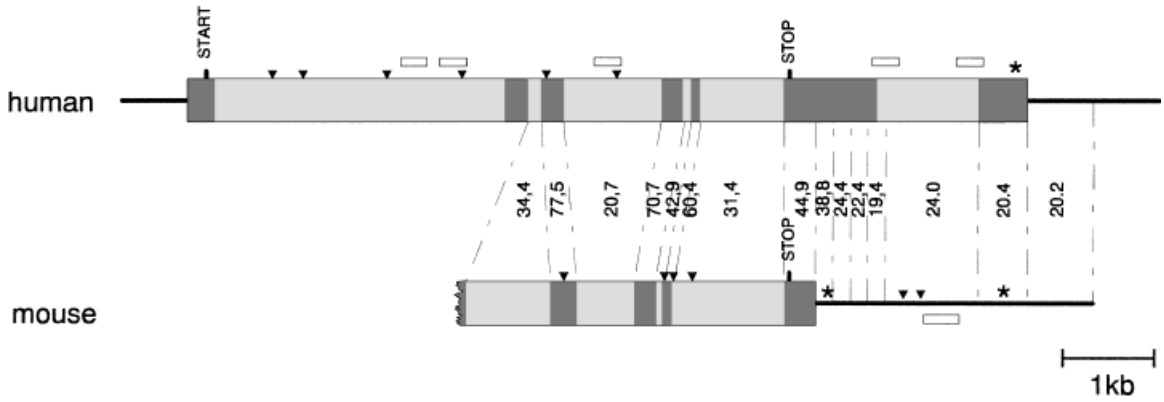

Fig. 2. Comparison of the HFE genes from man and mouse. Transcribed regions are indicated as shaded boxes. Exons are shaded in dark, introns are in light grey. The exon-intron boundaries were deduced by comparison of the newly analyzed genomic sequences with the published cDNA sequences [Feder et al., 1996, Acc. No.: U60319; Hashimoto et al., 1997, Acc. No.: U66849]: Repetive sequence elements (Alu in human, MT like in mouse) are indicated by open rectangles. Nucleotide sequence similarities in corresponding sections of the human and mouse HFE genes are shown in percent homology. Calculations were performed by the clustal method of the MegAlign programm of the DNAstar software. Compared sections are indicated by dashed lines. *Eco*RI restriction sites are shown as black arrowheads. Asteriks indicate sequences within the 3′ regions similar to the polyadenylation signals.

the human and mouse sequences revealed a conserved exon-intron structure between these species with the major exception of intron 2. This spans 208 bp in the human HFE gene whereas in mouse the intron sequence is 961 bp in length. Comparison of the amino acid sequences deduced from the cDNAs of man and mouse [Hashimoto et al., 1997] revealed eight additional amino acids at the exon2-exon3 border of the murine gene. These additional nucleotides may have been part of a major insertion increasing the size of intron 2 by about 750 bp. The amino acid sequence deduced from the rat cDNA sequence [Banasch et al., 1997] contains these additional amino acids, too.

The cDNA sequence of the mouse gene as published by Hashimoto et al. [1997] is significantly shorter than the corresponding cDNA in

man [Feder et al., 1996] and also shorter than the cDNA from rat [Banasch et al., 1997]. The 3′ end of the rat cDNA sequence shows high sequence similarity to the 3′ untranscribed region of the mouse gene and to the sequence of exon 6 of the human genomic sequence. On the other hand, the genomic sequence of the 3′ untranscribed region of the mouse gene beyond the cDNA data of Hashimoto et al. [1997] shows, at least in the first 200 bp, a significant sequence similarity with the corresponding region of the cDNA from man (Figs. 2 and 3). Thus, we may conclude that the published mouse cDNA is not full length or that alternative transcriptional termination sites may exist. In addition, the cDNA sequence of the mouse HFE gene contains no consensus sequence of a polyadenylation signal, and no polyA tail at the 3′ end was shown by Hashimoto et al. [1997]. From alignment studies of the rat cDNA sequence with the mouse and human sequences, we conclude that the mouse transcript is at least 160 bp longer than described by Hashimoto et al. [1997]. This is confirmed by the fact that a typical polyadenylation signal was found in the mouse genomic sequence at a position corresponding to the polyadenylation signal of the rat cDNA sequence (Fig. 3). An additional typical polyadenylation signal is located about 2 kb downstream of the deduced translational stop site of the mouse genomic sequence.

The exon-intron boundaries within the coding regions have the typical features, GT at the 5′ end of the intron and AG at the 3′ end [Breathnach and Chambon, 1981]. The deduced exon-intron boundary of the last intron, within the 3′ region of the human HFE gene, does not fit this rule. Within the coding region of the human and mouse gene all introns have a unique pattern of intron insertion within codons: the first nucleotide of the codon comes from the first exon and the other two nucleotides from the next exon.

In the human HFE gene, we identified 5 copies of the mobile repetitive elements of the Alu family (Fig. 2, open boxes). Three of these are located within introns. In intron 1 there are two copies with the same orientation separated by about 250 bp. The third element is located in intron 3. The second Alu repeat of intron 1 and that in intron 3 show an insertion by retrotransposition as the Alu elements are flanked by a polyA stretch and the flanking sequences show a rudimentary sequence similarity generated by duplication of the target sequences (Table I). The duplication of the target sequence is characteristic of insertions of mobile elements into staggered single-stranded nicks at different genomic locations [Weiner et al., 1986]. In the first Alu repeat element we found none of these features.

Two Alu elements are located at the exon6-intron6 and intron6-exon7 boundaries, respectively. Both 34 and 38 bp of the Alu sequences are within exons, the other portions are within introns. This means that both splice sites, the donor and the acceptor splice site, are generated by sequences of the Alu repeat elements. Alu sequences, which generate splice sites within genes, even in the coding region, are reported for several genes [Makalowski et al., 1994]. Since the element shows reverse orientations, the last 34 bp of exon 6 and the first 38 bp of exon 7 form a palindromic sequence, which is also present in the mRNA, with only 4 mismatches. Both repeat elements show the features for retrotranspositional origin, which are described above. The insertion sites are perfectly duplicated as shown in Table I.

The insertion of the two Alu elements within the 3′ region of the human HFE gene may have caused the imperfect exon-intron boundaries compared with these sites in the coding region and may explain the different length of the 3′ ends of the mRNA from man, mouse, and rat. In the genomic sequence of the mouse HFE gene, we did not identify a repetitive sequence element within the transcribed portion. Within the 3′ untranscribed region, we found a sequence element of the middle-repetitive MT type [Heinlein et al., 1986].

In the promoter region of the human HFE gene we found no consensus TATA sequence within the -25 region. We only find a TATA-like sequence (TAAAAA) at position -11 with respect to the transcription start site derived from the cDNA sequence. A CCAAT-box is found at position -40. Several consensus sequences of binding sites for homeobox-containing transcription factors are found upstream of this element. In particular, a binding site for: CdxA, a homeobox-containing protein from chicken, at position -70 to -76 [Margalit et al., 1993], a SRY binding site at position -184 to -190 [Pontiggia et al., 1994], and a NF-κB binding site at position -566 to -573 [Kunsch et al., 1992]. A typical poly-A signal site is present at position

Fig. 3. Sequence alignment of the 3′ untranslated portion of the cDNA sequence from mouse [Hashimoto et al., 1997] and rat [Banasch et al., 1997, Acc. No. AJ001517] and the genomic sequence of the human and mouse HFE gene. The sequence alignment starts with the stop codons of the HFE genes and extends until the end of the rat cDNA. The sequences of the polyadenylation signal are underlined. As indicated in Figure 2, the transcribed region of the human HFE gene continues about 2 kb beyond the 3′ ends of the two rodent RNAs shown here.

TABLE I. Duplication of the Integration Sites of the Alu Repeat Elements in the Human HFE Gene*

Intron 1	ACC**AAGG**C**A**-→-CTG**AAGG**A**A**
Intron 3	**AGAA**A**T**G**TCA**A-→- **AGAA**T**TCA**G
Exon 6–intron 6	**GAAAGTGAAGTA-→-GAAAGTGA-AGTA**
Intron 6–exon 7	**TCAAAAGAGTCTT-←-TCAAAAGA-GTCTT**

*Nucleotide sequences flanking the Alu repeat elements. Identical nucleotides are indicated with bold letters. Arrows indicate the orientation of the Alu elements.

+9590 to +9595, i.e., 15 bp upstream from the transcription end.

DISCUSSION

Previous studies have shown that the haemochromatosis candidate gene HFE (HLA-H) is located on the short arm of chromosome 6 in the region 6p21.3 and is associated with histone genes [Feder et al., 1996]. During our detailed analysis of the human histone gene cluster, which we had previously mapped to 6p21.3-p22 [Albig et al., 1993], we had isolated YACs spanning this region [Albig et al., 1997b]. Therefore, we could use the HFE (HLA-H) gene information to define the precise position of the haemochromatosis gene within the human histone gene cluster. We found the HFE gene in the vicinity of an H4 histone gene that is on one side in close association with the H1t gene [Drabent et al., 1991, 1995b] and, at a greater distance on the other side, with the H1.2 gene [Eick et al., 1989; Kardalinou et al., 1993; Albig et al., 1997b]. The localization and sequence of the human haemochromatosis gene were later confirmed by sequencing of a 240-kb region surrounding the haemochromatosis gene [Ruddy et al., 1997].

The organization of the haemochromatosis gene differs from the surrounding histone genes in several respects. First, in contrast to the HFE gene, none of the histone genes within the cluster containing 35 genes [Albig et al., 1997b] and within a further subcluster in the vicinity of the chromosomal marker D6S105 at the centromeric side of the major cluster [Albig and Doenecke, 1997] contains any introns. Second, all histone genes in this cluster have downstream processing sites that are involved in the S-phase dependent expression of the histone genes [Birnstiel et al., 1985; Schümperli ,1986; Marzluff and Pandey, 1988; Wang et al., 1996]. Thus, mRNAs derived from histone genes of this cluster are non-polyadenylated in contrast to the HFE (HLA-H) mRNA. Third, although the five classes of histone genes have class-specific promoter elements, a joint mechanism regulating the state of activation of the entire cluster may have been expected. Thus, the finding of a gene involved in entirely different functions, within the coordinately expressed histone gene cluster was not anticipated.

The HFE (HLA-H) gene is located downstream of an H4 gene associated with the H1t gene. We had previously shown that this association of the H1t gene with an H4 gene is common to man, mouse, and rat [Drabent et al., 1995b]. Furthermore, mapping of 6 murine H1 genes (H1.1-H1.5 and H1t) to 13A2.3 in the mouse genome [Drabent et al., 1995a] and of several rat histone genes to 17p12 [Walter et al., 1996] showed that histone gene clusters in these three mammalian species map to corresponding regions. The recent investigation of the major histone gene cluster of the mouse (at 13A2.3) by Wang et al. [1996] documented that the human and murine histone gene clusters not only map to corresponding regions, but also are similarly organized.

Recently, Hashimoto et al. [1997] have mapped the murine homologue of the haemochromotosis gene to 13A2.3 and deposited the cDNA sequence data in the EMBL nucleotide sequence database. Using this sequence information and our previous data on the association of the H1t gene and an H4 gene, we detected the murine haemochromatosis gene at the location predicted from histone gene cluster analogies between man and mouse. From these results we assume also that the rat hereditary haemochromatosis gene is located within the histone gene cluster, i.e., on chromosome 17 in the region 17p12.

As reported by Amadou et al. [1995], the region telomeric of the MHC in the human genome has been translocated to mouse chromosome 13 as a synteny unit. Thus, the HLA-type haemochromatosis gene, which is situated close to other HLA genes in the human genome, has been translocated away from the MHC region in the murine genome and probably in the rat genome, too. This further supports the notion that the human chromosome organization around the breakpoint represents the ancestral

one compared with this site in the murine (and rat, respectively) genome.

As discussed above, 3 of the 4 introns covered by the partial murine HFE clone are located at identical sites within the gene compared with the human HFE gene. Intron 2, however, is much longer in the murine haemochromatosis gene compared with the human HFE gene, and exon 3 abutting this intron has an extra eight amino acids. These may have been introduced in the context of an insertion event, which caused the increase in the intron 2 size. However, since the amino acid sequence deduced from the rat haemochromatosis cDNA sequence also contains these 8 additional amino acids, it may be an alternative posibility that these 8 amino acids have been lost in the human sequence due to a deletion event within exon 2 of the human gene.

Comparison of the domain structure of the human HFE protein, as indicated in the protein database (SwissProt, accession no.: Q30201), and the exon structure of the human HFE gene, as shown here, shows the typical distribution of MHC class I genes: each protein domain is encoded by one exon. The extracellular α1 domain is encoded by exon 2 and the last three amino acids of exon 1. The α2 and α3 domains of the protein are encoded completely by exon 3 and exon 4, respectively. Exon 5 codes for the connecting peptide, the potential transmembrane domain, and the first part of the cytoplasmic tail. The last 12 amino acids of the cytoplasmic tail are encoded by exon 6. This gene organization is similar in the mouse HFE gene, as far as revealed by our sequence data.

The murine clone does not cover exon 1, intron 1, and the major part of exon 2. Thus, we cannot compare the 5′ region of the genes and their promoters. Feder et al. [1996] detected HFE (HLA-H) transcripts in most tissues studied (except brain), although at different concentrations. The apparent tissue-dependent levels of expression of the human HFE (HLA-H) gene suggest that transacting factors may be involved in modulating expression of this gene. A TATA box was not found at the appropriate position around -25 of the transcription start site (as defined by the cDNA start site described by Feder et al. [1996]), but a CCAAT box was found at -40. This box showed an extension by CA (i.e., CCAATCA), which we have observed in most histone genes (Meergans et al., manuscript in preparation) and are found in some MHC class I genes [Srivastava and Lambert, 1991]. The promoters of these MHC class I genes also contain binding sites for NF-κB as does the HFE gene. The distance between these two elements is quite different in the MHC class I genes and the HFE gene; therefore, we cannot decide whether these sites have similar functions. It remains to be shown whether the transcription factor binding consensus elements, which we have found by computer searches of the HFE promoter sequence, are involved in the transcriptional regulation of this gene that is surrounded by histone genes that are expressed in a totally different functional context. We could not detect a sequence similar to the beta-globin analoguous promoter (β-GAP) sequences, which had been proposed by Rothenberg and Voland [1996] for a potential HFE gene. The authors detected this element in nonclassical class I genes that are proposed to be involved in iron absorption. The lack of this element is consistent with the finding that the mouse HFE gene is not located in the MHC class I region on chromosome 15, where all β-GAP containing genes are located.

In conclusion, our data describe the precise localization of the human and murine haemochromatosis gene within a large family of genes that differ from the HFE gene in organization, mode of expression, and functional context. The data further show that evolution has fully conserved this joint arrangement of functionally different genes. Further work on the S-phase dependent regulation of most histone genes within that cluster and future data on the control of expression of the HFE (HLA-H) gene will help to understand how the regulation of the haemochromatosis gene is coordinated with or insulated against the periodical, S-phase-dependent regulation of activity of histone genes surrounding the HFE gene.

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