Two Human Lysosomal Membrane Glycoproteins, h-lamp-1 and h-lamp-2, Are Encoded by Genes Localized to Chromosome 13q34 and Chromosome Xq24–25, Respectively*

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The lysosomal membrane plays a vital role in the proper function of lysosomes by sequestering numerous acid hydrolases, which are responsible for the degradation of foreign materials and for specialized autolytic phenomena (1). Glycoproteins localized primarily in the limiting membrane of lysosomes have been identified in human (2–5), mouse (12), rat (called lgp120) (13, 14), and chicken (called LEP100) (15) cells. The deduced amino acid sequences of these molecules share significant homology, and more than 50% identical residues can be found between species as distantly separated as human and chicken proteins. More recently, it has been shown that human lamp-1 has the disulfide arrangement different from that observed in members of the immunoglobulin superfam, despite the fact that it contains a functional hinge region (16). These results suggest that lamp-1 (and possibly lamp-2) re- related molecules represent a new family of membrane glycoproteins. Our results also showed that the amino acid sequence of human lamp-1 is more homologous to lamp-1 related molecules from other species than to human lamp-2 (4). Furthermore, lamp-1 and lamp-2 are immunologically distinguishable from each other (3, 5, 8). Based on these findings, we proposed that lamp-1 and lamp-2 diverged relatively early in evolution and that lamp-1 (and possibly lamp-2) structures have been strongly conserved during evolution. In this paper, we present a test of this hypothesis by identifying the chromosomal localization of lamp-1 and lamp-2 genes. The present results support our hypothesis, since the genes of the two glycoproteins have been found to be encoded on separate chromosomes.

EXPERIMENTAL PROCEDURES

Isolation of cDNA Probes—The plasmid p-hL1-15B containing a full-length cDNA of human lamp-1 mRNA was isolated from a human placental cDNA library as described (4). From this full-length cDNA, we made two smaller fragments of cDNA. One fragment, made by PstI digestion, stretches from 5' end to nucleotide 899, whereas the other human lamp-1 probe, made by PvuII digestion, encodes nucleotides 902–1462. The first probe encompasses the 5'-untranslated sequence plus sequences encoding amino acid residues 27 to 208. The second probe begins with the sequence encoding amino acid residue 211 and continues to the COOH-terminal, plus 24 nucleotides in the 3'-untranslated sequence. In addition to these probes, a cDNA clone isolated from a IMR-90 cDNA library (2) was also used. The cDNA, h-lamp A-4, encompasses the lamp-1 polypeptide coding region flanked by a short 5'- or 3'-untranslated sequence, lacking a poly(A) tail. This cDNA, however, has a deletion of 83 base pairs (nucleotides 216–290) from the full-length cDNA (2, 4). A cDNA fragment containing nucleotides 530–1543, was obtained by EcoRI digestion of the human cDNA lamp-2 cDNA clone isolated from IMR-90 eDNA library (4). This cDNA fragment, h-lamp-2-h,
contains sequences corresponding to amino acid residue 117 and runs the COOH terminus of the polypeptide plus 21 nucleotides of 3'-untranslated region. All these cDNA probes were inserted in the plasmid Bluescript (Stratagene) utilizing the appropriate sites. The plasmids were purified by centrifugation in two cesium chloride gradients and the whole purified plasmids were used as probes.

In Situ Chromosome Hybridization—In situ hybridization was carried out on chromosome preparations obtained from phytohemagglutinin-stimulated human lymphocytes cultured for 72 h. The conditions for labeling of probes and hybridization and washing were as described previously (17). After coating with nuclear track emulsion, (Kodak NTBZ), the slides were exposed for 19 days at 4 °C and were developed. To avoid any slipping of silver grains during the banding procedure, chromosome spreads were first stained with a buffered Giemsa solution and metaphases photographed. R-banding was then performed by the fluorochrome-photolysis-Giemsa method and metaphases rephotographed before analysis.

Southern Blot Hybridization-K562 cell genomic DNA was digested with restriction enzymes using an excess of enzyme for approximately 4 h at 37 °C using the conditions recommended by the supplier (Bethesda Research Laboratories) (18). Ten-μg equivalents of digested DNA were resolved by 0.8% agarose gel electrophoresis. The gel was treated successively with 0.2 N HCl at room temperature for 15 min, alkali-denatured, neutralized, and transferred to nylon filters (Nytran, purchased from Schleider and Schuell), as described by Southern (19). The acid treatment was included in order to insure the efficient transfer of high molecular weight DNA.

Hybridization of the filters was performed for 16 h at 65 °C in 5 X SET, 0.5% sodium dodecyl sulfate, 1 × Denhardts, 10% dextran sulfate (for details of the solutions see Ref. 20), and 2–3 ng cDNA probes/ml. cDNA probes were labeled with 32P by nick translation or random oligonucleotide primed methods (21). The filters were then washed with 2 × SET, 0.5% sodium dodecyl sulfate at 65 °C until the background count, monitored by a hand-held Geiger counter, disappeared (4).

The probe for lamp-1 was made from the plasmid Bluescript harboring the 2.4-kilobase pair full-length cDNA of h-lamp-1, hL115B (4). Similarly, the probe for h-lamp-2 was made from the plasmid Bluescript, harboring a full-length h-lamp-2 cDNA (4). This plasmid also contains flanking Axl11 sequence since the Kpnl and SaeI sites in the vector were used to excise the cDNA from the Axl11 clone (4). These Bluescript plasmids were linearized by PstI (for lamp-1) or BamHI (for lamp-2) digestion before labeling.

RESULTS AND DISCUSSION

Southern Blot Analysis of Human lamp-1 and lamp-2 Genes—We have characterized human lamp-1 and lamp-2 genes by Southern blot of genomic DNA digested with Axl1, BamHI, BglII, EcoRI, HindIII, and SstI. Two blots were made and each blot was hybridized with a cDNA probe for h-lamp-1 or h-lamp-2.

Fig. 1 shows that a small number of bands were detected after digestion with each enzyme in both lamp-1 and lamp-2 genomic fragments. During these experiments, we initially encountered difficulty in detecting bands on lamp-2 genomic fragments when DNA was not treated with acid before denaturation. This may be due to the fact that a majority of h-lamp-2 genomic fragments using h-lamp-2-h as a probe are of high molecular weights. Including acid treatment improved the detection of genomic fragments, as shown in Fig. 1B. However, the signals obtained were still so weak that specific hybridization could not be obtained in experiments designed to analyze genomic DNA from somatic hybrid cells. If the stringency of the conditions for hybridization and washing was reduced, nonspecific bands became rather prominent. Nevertheless, Fig. 1B shows that the h-lamp-2 probe hybridizes to a very small number of large genomic DNA fragments. These data indicate that h-lamp-2 gene is present as a single copy in the human genome.

Fig. 2. Distribution of labeled sites on chromosome 13 (A) and chromosome 12 (B) obtained by using h-lamp A-4 (h-lamp-1) probe. The peak of hybridization occurs on band q34 of chromosome 13 (A) and on band p133 on chromosome 12 (B).
We have observed that the restriction fragments containing 3'-untranslated region give strong signal when total genomic DNA is analyzed by a full-length cDNA during the studies on glycophorin. We believe that this is because 3'-untranslated region is often coded by a relatively large exon (for example, see ref. 22), which would give strong signal after hybridization. Since cDNA probe of h-lamp-2 lacks 3'-untranslated sequence, this may be the reason why the signal of genomic hybridization with h-lamp-2 was weak.

The full-length h-lamp-1 probe detected a small number of DNA fragments with greater intensity than the h-lamp-2 probe. However, a number of fragments were relatively small, and these data did not necessarily suggest that sequences corresponding to the h-lamp-1 cDNA are present in more than one site in the genome. Another possibility suggested by our data is that a single copy of the h-lamp-1 exists with the coding regions divided by a number of large introns, which could contain restriction enzyme recognition sites.

Localization of the h-lamp-1 Gene to Chromosome 13q34—To investigate further the number of gene copies of h-lamp-1, and to determine the chromosomal localization of human lamp-1 full-length gene, we hybridized human lamp-1 cDNA (h-lamp-1-A-4) to normal metaphase chromosomes. This resulted in specific labeling of chromosome 13. Of 200 metaphase cells examined from this hybridization, 382 silver grains were associated with chromosomes and 106 of these (27.7%) were located on chromosome 13. The distribution of grains on this chromosome was not random, and 84% of them were mapped to q33→q34 region of chromosome 13 long arm with a maximum in the 13q34 band (Fig. 2A).

In addition to this, a minor hybridization site was detected on chromosome 12 (10.2% of total grains) and 61.5% of the...
grains on chromosome 12 to the p123 → p133 band (Fig. 2B). Fig. 3 illustrates the histogram of the grain distribution, showing that the major site is at chromosome 13q34, whereas the minor site is at chromosome 12p133.2 We went further to clarify the gene localized at 12p133 by making two different cDNA probes available, which represent the NH2-terminal half and the COOH-terminal half of the h-lamp-1 molecule, respectively (see “Experimental Procedures”). Both probes, however, provided essentially the same results presented above (data not shown).

These results suggest that the gene localized to chromosome 13q34 encodes genuine h-lamp-1, whereas the gene localized at chromosome 12p133 probably encodes for a sequence homologous to h-lamp-1. This homologous sequence could be either an ancestral gene for h-lamp-1 and possibly for lamp-2 (see below), pseudogene of h-lamp-1, or duplicated and translocated h-lamp-1 gene. In other studies, two or more different sites have been reported for chromosomal localization for genes such as Ha-ras (23, 24), Lu-L-fucosidase (25, 26). experiments similar to those described above were carried out using a probe containing the 3'-half of h-lamp-2 cDNA (nucleotides 530 to 1343). This probe specifically hybridized to chromosome X (24.8% of total grains) and 78% of the grains chromosome X, bands q24-q25.

The results therefore indicate that the gene detected at chromosome 12p133 by the h-lamp-1 probe encodes a sequence very homologous to h-lamp-1, and this region must have more identical sequences with h-lamp-1 than h-lamp-2. These results reinforce our idea and suggest that lamp-1 and lamp-2 are distinct from each other also at the level of genomic structures.

The present study clearly indicates that human lamp-1 and lamp-2 genes are localized on entirely different chromosomes despite the fact that they share homologous structures. As described previously (4), the amino acid sequence of human lamp-1 is more homologous to lamp-1 related molecules from other species than it is to human lamp-2. These data, together with the results presented in this paper, strongly suggest that lamp-1 and lamp-2 diverged from an ancestor gene early in evolution, perhaps as soon as eukaryotic cells acquired lysosomes as subcellular compartments, but that the structure of lamp-1 (and possibly lamp-2) has been strongly conserved during evolution. It is therefore highly possible that lamp-1 and lamp-2 have distinctly different functions. The present results now provide a basis to correlate the cause of a particular disease to the defect in lamp-1 or lamp-2. If a gene for a particular disease is mapped to the same chromosomal location as the lamp-1 or lamp-2 gene, it will be important to determine if such disease is caused by the defect in lamp-1 or lamp-2 molecules.

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REFERENCES

Fig. 5. Distribution of labeled sites on chromosome X by using h-lamp-2·h probe. The peak of hybridization occurs on bands q24 and q25 of chromosome X.

3 These results were confirmed by Southern hybridization of genomic DNA isolated from cell hybrids: S. Naylor and M. Fukuda, unpublished results.

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Two human lysosomal membrane glycoproteins, h-lamp-1 and h-lamp-2, are encoded by genes localized to chromosome 13q34 and chromosome Xq24-25, respectively.

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