The Gene For the Major Intrinsic Protein (MIP) of the Ocular Lens is Assigned to Human Chromosome 12cen-q14

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The gene for the human major intrinsic protein (MIP) of the ocular lens fiber membrane has been assigned to region cen-q14 of chromosome 12 through the use of somatic cell hybrids containing the whole chromosome and parts of human chromosome 12. Invest Ophthalmol Vis Sci 27:1351-1354, 1986

The major intrinsic protein (MIP) of the ocular lens fiber membrane is an abundant protein that appears during differentiation of the ocular lens and has a molecular weight of 26,000 daltons. The biological function of this protein is not known, but evidence suggests that it may be a major component of lens fiber gap junctions.1,2 Consistent with this notion is the distribution of MIP throughout the fiber cell plasma membrane, including the gap junctions. Its relation to disease is not known, but, conceivably, mutation of the structural gene could lead to genetic disease of the lens of the eye. The chromosomal location of the human gene for MIP has been previously unknown. The bovine cDNA for this protein has recently been isolated and characterized.3 Using this bovine probe for MIP in the study of its segregation in mouse-human somatic cell hybrids, we have assigned this gene to the cen-q14 region of the long arm of human chromosome 12.

Materials and Methods

Somatic cell hybrids were derived from the fusion of thymidine kinase (TK) deficient mouse cells (B82, GM0347A) and normal human male fibroblasts (IMR91), both obtained from the Mutant Cell Repository (Camden, NJ). The cells were fused in a mixed monolayer using a 50% solution of polyethylene glycol (mol. wt. 1,000) in a balanced salt solution. Following a 24-hr culture period, the cells were added to multiple independent culture dishes containing HAT medium and ouabain.7 Multiple independent hybrid clones were isolated and a preliminary cytogenetic analysis was done on 10 Q-banded photographed chromosome metaphases. Sixteen hybrid clones were selected from an initial set of 40 clones, based on growth characteristics, human chromosome content, and a lack of detectable human chromosome rearrangements. As expected with this type of procedure, the hybrid clones contained different human chromosome content. The clones were then grown in multiple dishes, pooled, and cell pellets were prepared for DNA extraction. From the same pooled cells of each clone, an analysis of chromosome content was made on a minimum of 30 Q-banded photographed metaphases per hybrid clone. Because these mouse-human hybrids do not retain human chromosome 9, DNA from a Chinese hamster-human hybrid clone selectively retaining 9pter-9q34 by virtue of an X/9 translocation was also analyzed.

Seven additional Chinese hamster-human hybrid clones selectively retaining chromosome 12 or parts of chromosome 12 were used to regionally map the gene for MIP on chromosome 12. The formation of these clones and their characteristics have been published previously as follows: E4E;9,10 37A9, 16-16, 16-43 and 16-33;11,12 and J532-1a and 480-22A.13 The deleted parts of chromosome 12 and the presence or absence of MIP are noted in Figure 1.

DNA from the parental cell lines and somatic cell hybrid clones was purified from isolated nuclei by incubation in 10 μM EDTA pH 8.0, 0.2% SDS, 600 μg/ml proteinase K (Sigma, St. Louis, MO) at 37°C for 24 hr, followed by phenol extraction and ethanol precipitation.

The bovine probe, 789 bases long, for the major intrinsic protein3 cross-hybridizes with human DNA. This probe was used to detect the presence or absence...
of the human MIP DNA. The enzymes were obtained from Bethesda Research Laboratory (Gaithersburg, MD) and radiolabelled nucleotides were obtained from Amersham Corporation (Arlington Heights, IL). The MIP probe was radiolabelled with \( ^{32} \text{P} \) to a specific activity of approximately \( 1-3 \times 10^9 \text{cpm/\mu g} \) by random priming.\(^{15} \) Genomic DNAs from the parental cell lines and the hybrid clones were digested with the restriction endonuclease Hind III (8 U/\mu g). Approximately 10 \mu g grams of DNA from each sample were electrophoresed through a 1.2% agarose gel in TAE (40 mM Tris acetate, 1 mM EDTA pH 7.4) buffer and transferred by blotting to nitrocellulose by the method of Southern.\(^{16} \) Hybridization was performed in 45% formamide, 4.6\text{X} \text{SSC}, 5\times \text{Denhardt's solution}, 20 \text{mM NaH}_2\text{PO}_4 \text{pH 6.5, 250 \mu g/ml denatured salmon sperm DNA and 10\% dextran sulfate for 16 hr at 42°C with shaking. The filter was then washed twice, first in 2\text{X} \text{SSC, 0.1\% SDS and then in 0.1\text{X} \text{SSC, 0.1\% SDS, each time for 20 min at 55°C. The filter was dried briefly and exposed to Kodak (Rochester, NY) XAR-5 Xray film.}

Results

The DNA probe detects a 6.9 kb band in the human genomic DNA following digestion with Hind III (Fig.

Fig. 1. Diagram of chromosome 12 with the remaining part of the deleted chromosome 12 noted for each of seven somatic cell hybrids used for regional mapping of the MIP. Deletion of chromosome 12 for each of the hybrids is: E4E, none; 37A9, pter-p1205; 16-33, short arm translocated to hamster chromosome; 16-43 q14.3-qter, short arm breakpoint not clear; 16-16, 14.3-qter; 480-22a, long arm; and J532-1A, short arm. The presence (+) or absence (−) of the human MIP for each hybrid is noted at the bottom. These results regionally map MIP to 12cen-12q14.3.

Fig. 2. Southern blots for MIP chromosome assignment: channel F is mouse parent; channel G is human parent with 6.9 kb band. Channels A–E are results from somatic cell hybrid clones with A (84-4) and B (84-34) negative for human band and C (84-2), D (84-7), and E (84-20 positive for human band. DNA was cut with Hind III.
Table 1. Segregation of MIP gene with human chromosomes in cell hybrids

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No. of discordant hybrids

| 10 | 11 | 9  | 5  | 5  | 6  | 7  | 11 | 9  | 6  | 0  | 10 | 7  | 9  | 8  | 5  | 6  | 11 | 6  | 5  | 7  | 11 | 10 |

* + indicates presence of the MIP sequence in the hybrid clone determined by the presence of the human band; - indicates absence of the gene.

** + indicates presence of the human chromosome in greater than 30% of metaphases analyzed; (+) indicates presence of the chromosome in 10-30% of metaphases analyzed; - indicates absence of the chromosome.

2). This band is easily distinguishable from the 3.6 and 4.5 kb bands from the mouse genomic DNA (Fig. 2). Table 1 shows the presence/absence of the human MIP band in relation to the human chromosome content of the somatic cell hybrids used for the general mapping. Eleven of the hybrids were positive for the human MIP, while five were negative. Correlation of the human band with the human chromosome content of these hybrid clones shows that the MIP DNA resides on chromosome 12. There were no discordant clones for the co-segregation of the MIP and chromosome 12. All other chromosomes showed at least five or more discordancies. None of the mouse-hybrid clones retains the human chromosome 9; analysis of a single Chinese hamster-human hybrid clone selectively retaining most of chromosome 9 was negative for the presence of human MIP.

To regionally localize the MIP gene on chromosome 12, DNA from seven Chinese hamster-human hybrid clones retaining different parts of chromosome 12 were
analyzed using the same MIP probe. The results of these studies are seen in the Southern blot of Figure 3, and the correlation of the human MIP with the specific remaining parts of chromosome 12 are summarized in Figure 1. These results permit the assignment of the gene for MIP to the human chromosome 12cen-q14.3.

### Discussion

With recent technical developments, human gene mapping has recently shown rapid progress. The present paper is an example of this, and illustrates the use of somatic cell hybridization with a specific DNA probe to map the gene for the major intrinsic protein (MIP) to the cen-q14.3 region on the long arm of human chromosome 12. This approach utilizes the phenomenon of the loss of human chromosomes in somatic cell hybrids which have been formed with an established mouse line. The presence of the human DNA in the somatic cell hybrids is based upon the use of a bovine DNA probe which cross-hybridizes with the human MIP gene. Evaluation of the presence or absence of the human DNA for the MIP gene is correlated with the presence of the human chromosome content of the somatic cell hybrids. This demonstrates that the MIP gene can be assigned to chromosome 12. In a similar fashion, using the same probe in somatic cell hybrids between Chinese hamster and human cells retaining different parts of chromosome 12 permit the assignment of the MIP to the region on chromosome 12 between the centromere and band q14.3 on the long arm of chromosome 12. Such information is important for the following reasons. First, the results are consistent with the presence of only a single gene for the MIP, which is compatible with the results of Gorin et al. Such information is also important in looking for genetic heterogeneity in which a clinical phenotype may result from mutation in different genes. This has important implications for both diagnosis and genetic counseling. For those persons interested in evolutionary relationships, it permits correlation with gene mapping information in other species, such as rodents, to determine the degree of conservation in gene mapping relationships between species.

An extension of the isolation of the DNA probes for genes such as the MIP makes it possible to evaluate whether the gene is related to a specific disease. Specifically, in the case of the MIP, whose function is not fully understood, the type of disease which could result from dysfunction is unknown; and, at the present time, no known lens disease gene has been mapped to human chromosome 12. Through the use of restriction endonucleases in identifying restriction fragment length polymorphisms (RFLPs), it is possible to carry out family studies in relation to specific diseases of the lens of the eye, such as genetically determined cataracts. Demonstration of co-segregation of the inherited disease with the presence of the specific RFLP in the affected family may make possible the identification of the abnormal function of the gene in question and, by inference, its normal function. Such information may give insight into the pathophysiology of the disease and, eventually, possible intervention to prevent or reduce the expression of the mutant gene.

**Key words:** major intrinsic protein (MIP), DNA probe, somatic cell hybrids, human chromosome 12, lens

### References