Macrophage scavenger receptors (MSR) mediate the binding, internalization, and processing of a wide range of negatively charged macromolecules. Functional MSR are trimers of two C-terminally different subunits that contain six functional domains. We have cloned an 80-kilobase human MSR gene and localized it to band p22 on chromosome 8 by fluorescent in situ hybridization and by genetic linkage using three common restriction fragment length polymorphisms. The human MSR gene consists of 11 exons, and two types of mRNAs are generated by alternative splicing from exon 8 to either exon 9 (type II) or to exons 10 and 11 (type I). The promoter has a 23-base pair inverted repeat with homology to the T cell element. Exon 1 encodes the 5'-untranslated region followed by a 12-kilobase intron which separates the transcription initiation and the translation initiation sites. Exon 2 encodes a cytoplasmic domain, exon 3, a transmembrane domain, exons 4 and 5, an α-helical coiled-coil, and exons 6-8, a collagen-like domain. The position of the gap in the coiled coil structure corresponds to the junction of exons 4 and 5. These results show that the human MSR gene consists of a mosaic of exons that encodes the functional domains. Furthermore, the specific arrangement of exons played a role in determining the structural characteristics of functional domains.

Macrophage scavenger receptors (MSR) are trimeric glycoproteins, which are implicated in the pathologic deposition of cholesterol during atherogenesis through the uptake of modified low density lipoproteins (LDL) and also in the host defense against pathogenic organisms through the recognition of bacterial endotoxin precursors (1-6). MSR mediate multiple functions. One of the most characteristic functions of MSR is its recognition of an extraordinarily wide range of ligands, including modified LDL (e.g. acetyl-LDL, oxidized-LDL), modified proteins (maleylated bovine serum albumin), polynucleic acids (poly(I), poly(G)), carbohydrate (fucoidan), and other macromolecules (1). In order to mediate its role, the receptor must be processed and folded properly (7, 11) and expressed only on the cell surface of macrophages (8). After binding their ligands, the receptors gather in the coated pits, are internalized, and appear in certain vacuoles which lack acid phosphatases, probably endosomes of macrophages. They, then, dissociate their ligands and return to the cell surface (8, 9). One of these multifunctional receptors is a well studied LDL receptor. In case of the LDL receptor, the gene consists of a mosaic of exons encoding each functional domain (10). The functional MSR protein is a trimer containing two different C-terminal subunits. Each subunit consists of six unique functional domains (2-4, 11).

In order to elucidate the genetic bases of this complex multifunctional receptor, and to study its regulation of gene expression, we have cloned and characterized the human MSR gene. In this paper we report 1) the complete nucleotide sequences of cDNAs, 2) the cloning and characterization of the whole MSR gene, 3) analysis of the 5'-flanking region, 4) determination of exon/intron organization and its relationship to protein structure and function, 5) chromosomal localization as determined by in situ hybridization, 6) the presence of three common restriction fragment length polymorphisms (RFLPs), and 7) the placement of the locus on the genetic linkage map of human chromosome 8.

MATERIALS AND METHODS

Cloning of Full-length cDNA.―In order to obtain the full-length cDNA, we screened a cDNA library of a human monocytic leukemia cell line THP-1 treated for 3 days with 200 nM of phorbol 12-myristate 13-acetate (PMA) (4), using fragments from the 5' region of human type I and II MSR cDNAs, phSR1 and phSR2 (4), as probes. Three cDNA clones were found to contain an additional 20-bp sequence beyond the 5' ends of phSR1. Screening of this library with type-specific restriction fragments from the 3' regions of phSR1 and phSR2, respectively, as probes, resulted in isolation of clones containing additional sequences from the 3'-non-coding regions. Since these clones did not contain 3' ends of cDNAs including the poly(A) signal and poly(A) tail, we performed an amplification of cDNA ends by the RACE method (12). RNA of PMA-treated THP-1 was used as
a template for the polymerase chain reaction (PCR) using an oligo(dT)-linker primer in combination with primers specific to either the type I or II 3' non-coding regions. PCR products were analyzed by Southern blot hybridization with an internal oligonucleotide as a probe. A hybridizing DNA band was cloned, and three independent clones were sequenced to exclude PCR artifacts.

**Genomic Cloning**—A cosmide library containing human genomic DNA partially digested with Sau3A was constructed in a cosmid vector pWE16 by a method previously described (13). Approximately one million recombinants were screened with inserts of cDNA phSR1 and phSR2 as probes. Of the 18 positive clones, six were isolated. A representative clone, cMSR-15, -32, -53, -35, -40, and -44 were chosen for further analyses. Clones were aligned by restriction mapping and Southern blot hybridization with oligonucleotide probes synthesized from cDNA sequences. This analysis showed that three clones, cMSR-44, -10, and -32, overlapped with each other, and that another set of three, cMSR-33, -40, and -44, also overlapped with each other, although separately. Thus, two cosmide contigs were isolated in the MSR genomic region (Fig. 1). A gap between the two contigs could not be isolated after several attempts to screen the cosmide library with region-specific probes. The size of the gap was estimated by Southern blot analysis of genomic DNA using end probes of cMSR-32 and cMSR-33. Both probes hybridized to a 15.5-kb EcoRI fragment and a 14-kb BamHI fragment, indicating that the gap between these cosmids was located in these fragments. These two genomic fragments were cloned in a FIX and λ DASH vectors (Stratagene) from genomic DNA completely digested with EcoRI and BamHI, respectively. A clone, AE10, containing an EcoRI-digested insert and a clone, MB10, containing a BamHI-digested insert, were chosen for further analysis from positive clones (Fig. 1). Genomic clones were subcloned in pBluescript II (Stratagene) after digestion with EcoRI, XbaI, HindIII, or BglII. Nucleotide sequences of exons and their boundaries were determined on these subclones. From the murine genomic DNA library, a λ phage clone encoding an α-helical coiled-coil domain was cloned.

**Determination of Transcription Initiation Site**—Primer extension reactions (14) were carried out with 30 pg of poly(A)+ RNA obtained from THP-1 treated with PMA (2, 4) or human liver. A single-stranded oligonucleotide, 5'-d(GTCCTAAAGAAAGCAGC)-3', which is complementary to the sequence of the cDNA, was used as a primer. For an RNase protection assay (15), a 206-base DNA fragment corresponding to the genomic DNA sequence surrounding the transcription initiation site was amplified with primers, forward-AGAGTGAAGTCTGAC and backward-GTCTCTAAAGAAACGACGT-3', which is complementary to the sequence of the 5' end of cDNA (4). A probe was generated by PCR using T7 RNA polymerase directed plasmid and the DNA subcloned. A hybridization was performed with the LINKAGE program package (17). The genetic map was constructed from the reference families by using the GMS (Gene Mapping System) algorithm, as described previously (18).

**RESULTS AND DISCUSSION**

**Complete Structure of Type I and II MSR cDNAs**—The cDNAs clones isolated by the combination of the screening of the PMA-treated THP-1 cDNA library and the RACE method covered the entire sequences of the 3' end of type I and II MSR cDNAs. Results of the primer extension and RNase protection assay (see below) were taken into consideration for determination of the cDNA 5' ends. Type I MSR cDNA consists of 122 bp of 5'-untranslated region, 1353 bp of coding region, and 2204 bp of 3'-untranslated region as determined by the RACE method. There is a sequence, ATAAA, 15 bp upstream from the polyadenylation site. The length of type II MSR mRNA determined by Northern analysis also corresponds to the total length of the type II MSR cDNA plus poly(A) tail.

- **Type I** and **II MSR cDNA** have an identical sequence from the 5' terminus to nucleotide number 1155, but each has their unique 3' coding and untranslated sequence thereafter. This strongly suggests that the type I and II cDNAs are generated from a single gene by alternative splicing processes involving the 3' region of the gene.

**Cloning of Human MSR Gene**—A 120-kb genomic region containing the entire human MSR gene was cloned as a single contig consisting of six overlapping cosmids and two λ phage clones (Fig. 1). The length of the MSR gene is approximately 80 kb, which is about 20 to 30 times the size of type I or II cDNA, respectively. It consists of 11 exons interrupted by 10 introns (Table I). Exons 1 through 8 and 10 range from 54 to 413 bp in size, while exons 9 and 11 are relatively long, being 1676 and 2335 bp, respectively. Sequences at the exon-intron junctions for all 10 introns are compatible with the consensus sequences for the splicing junctions, including AG-GT (19). Comparison of the gene sequence with two cDNA sequences revealed that exon 1 through 8 encode the 5' DNA region common to both type I and II cDNAs, exon 9 encodes the C-terminal coding region and the 3'-untranslated region specific to type II cDNA, and exons 10 and 11 encode the C-terminal coding region and the 3'-untranslated region specific to type I cDNA (Fig. 1). This structural feature demonstrates that type I and II cDNAs are generated from a single MSR gene by alternative splicing from the last common exon (exon 8) of the MSR gene to either exon 9 (for type II cDNA) or to exon 10 and 11 (for type I cDNA). Both exon 9 and exon 11 possess polyadenylation sites for type I and II mRNAs, respectively. The presence of a single MSR gene in human genome was also verified by Southern blot hybridization of genomic DNA with several probes, each containing single exons of the MSR gene. Each probe detected a single band in several restriction enzyme digests, indicating the presence of a single MSR gene in the human genome (data not shown).

**Characterization of the 5' End of MSR Gene**—The site of transcription initiation in the MSR gene was determined by primer extension analysis and RNase protection assay using poly(A)+ RNA obtained from PMA-treated THP-1 and human liver tissue. The size of the 5'-non-coding region determined by primer extension analysis is 122 bp (Fig. 2). There is an intron which splices the 5'-non-coding sequence. Since the 5'-non-coding region is not fully covered by the cloned cDNAs, we confirmed the transcription initiation site by RNase protection assay. As can be seen in Fig. 3, the size of the probe protected from RNase digestion was the same as that determined by primer extension analysis. The protection was specific to mRNA of macrophages (Fig. 3, lane 1); mRNA from HEL cell (cultured human erythroblast cells) could not protect the probe (lane 2).

The nucleotide sequence of the 5'-flanking region is shown in Fig. 4. There are two AT-rich sequences, TATTGAAAA and ATTAAGAAA, that might serve as TATA boxes (20, 21) between 18 and 37 bp upstream from the transcription initiation position. We did not find the sequence CCAAT (21). The presence of two TATA-like sequences and the lack of a
Human Macrophage Scavenger Receptor Gene

**FIG. 1. Structure of the human MSR gene.** The upper numbers refer to the positions of exons. Exon 9 encoding the nucleotide sequence specific to type II cDNA is indicated as a white box, and exons 10 and 11 encoding type I receptor-specific sequence are indicated as boxes with stripes. The bold lines indicate the position of clones. XhoI sites and the XhoI fragment sizes are shown.

**TABLE I**

| Exon no. | Exon size | Sequence at exon-exon-intron junction | Amino acid interrupted |
|----------|-----------|--------------------------------------|------------------------|
|          |           | 5' Splice donor 3' Splice acceptor     |                        |
| 1        | 118       | GGAGGAAG gtaaaga ttcataag ATG ATG     | 5'-Noncoding           |
| 2        | 107       | CCT CCG A gtaagta ttcatta AT CCT AAA  | Asn (35)               |
| 3        | 114       | GCG GCA G gtaacgt ttcataa CTCAA CTC   | Ala (73)               |
| 4        | 413       | A CAA GAG gtaagag ttcataa GAA ATC AG  | Glu-Glu (210–211)      |
| 5        | 187       | ATT CAA G gtaaagat ttcataa GT CCT CCT | Gly (273)              |
| 6        | 81        | CCA ATA G gtaaggat aatgata GT CCT CCG | Gly (300)              |
| 7        | 81        | AGG CCA G gtatctac ttcataa GA AAT TCT | Gly (327)              |
| Type II  |           |                                      |                        |
| 8        | 54        | ACA TTA A gtaagta gttcag GA CCA GT A  | Arg (345)              |
| 9        | 1676      |                                      |                        |
| Type I   |           |                                      |                        |
| 8        | 54        | ACA TTA A gtaagta ttcataa GT CCA TTT | Thr (345)              |
| 10       | 189       | GCA CAA G gtaagta gttcag GT ACT GGT   | Gly (408)              |
| 11       | 2335      |                                      |                        |

**FIG. 2. Site of transcription initiation in the human MSR gene as determined by primer extension analysis.** The primer-extended products obtained using mRNA of THP-1 treated with PMA (right lane) were subjected to electrophoresis through a 6% polyacrylamide 50% urea gel and detected after autoradiography. The TATA box-like sequences and the position of transcription initiation site are indicated.

**FIG. 3. Site of transcription initiation as determined by RNase protection analysis.** The probe fragments protected by poly(A)" RNA of THP-1 treated with PMA (lane 1) or control poly(A)" RNA obtained from HEL cell (lane 2) were analyzed on 6% polyacrylamide 50% urea gel electrophoresis and were compared with the adjacent sequence ladder obtained with the same primer (31) and a subclone of genomic DNA containing the 5'-flanking region. The antisense sequence is indicated.
Human Macrophage Scavenger Receptor Gene

Fig. 4. Nucleotide sequence of the 5'-flanking region of the human MSR gene. Nucleotide position of the transcription initiation site is assigned to position +1. Boxes indicate the TATA box-like sequences. Solid underlines indicate additional inverted repeat sequences. Dotted underlines indicate 23-bp inverted repeat sequences.

Fig. 5. Relationship of exon organization and functional domains. The numbers in the boxes refer to exon numbers. The type I transcript consists of the common exons 1-8 and type I-specific exons 10 and 11. The type II transcript consists of exons 1-8 and type II-specific exon 9. The positions of the gap within the coiled-coil structure, which corresponds to the junction of exons 4 and 5, and putative ligand-binding domain, which is encoded by exon 8, are indicated above the functional domains.

Fig. 6. α-Helical coiled-coil structure of MSR in various animals. Amino acid sequences in the α-helical coiled-coil structure were determined in human, bovine, murine (deduced from the nucleotide sequence of cDNA for P388D1 MSR), and rabbit (deduced from the nucleotide sequence of rabbit lung type I MSR cDNA). The apparent heptad repeats of hydrophobic amino acids are indicated by bold letters. The possible N-glycosylation sites are underlined. The histidine interrupting heptad repeats of hydrophobic amino acids are indicated by bold and italic letters.

Table II
Three RFLPs at human MSR locus

| Clone name | Probe fragment | Enzymea | Allele size | Allele frequency | Heterozygosity |
|------------|----------------|---------|-------------|------------------|---------------|
| cMSR-32    | E-H 0.9        | MspI    | kb          | 6.3              | 0.71          | 0.47          |
|            |                |         |             | 3.1              | 0.10          |              |
| cMSR-32    | E-H 0.9        | BamHI   | 14.0        | 0.78             | 0.27          |
| cMSR-35    | Hind 9.0       | HindIII | 13.0        | 0.20             | 0.26          |

Table Continued

| Clone name | Probe fragment | Enzymea | Allele size | Allele frequency | Heterozygosity |
|------------|----------------|---------|-------------|------------------|---------------|
| cMSR-35    | Hind 9.0       | HindIII | 9.0         | 0.80             |               |

a E-H 0.9 denotes a 0.9-kb EcoRI-HindIII fragment, and Hind 9.0 denotes a 9 kb HindIII fragment.
b Enzyme used for the digestion of genomic DNA.

CCAAT box was similar to the structure of the promoter of the human LDL receptor gene (10). In contrast, the consensus sequence of a sterol-responsive element which mediates the down-regulation of gene expression by sterol in the LDL receptor gene (22) was not found in the MSR promoter region. This result is consistent with the finding that MSR activity is not regulated by cellular sterol content, which leads to the accumulation of cholesterol and foam cell formation during atherogenesis (1). There is a 23-bp inverted repeat sequence at -592 to -570, and at -424 to -402 (Fig. 4, underlined). The region surrounding this 23-bp also has additional inverted sequences (Fig. 4, dotted underline). This inverted repeat sequence contains a subsequence GGGATTACA which is highly homologous to the consensus T cell element GGGPuTTT(C/A)A, which mediates the T cell-specific induction of the interleukin 2 gene by phorbol ester (23). The expression of the MSR protein is limited to macrophages and related cells (6, 7), and in THP-1 cells the expression is induced by phorbol ester treatment (2, 11). A consensus for the AP-1 binding site, TGA(G/C)TCA, which mediates the phorbol ester-inducible enhancer element (24), was not found in this region. The expression of the MSR gene reaches the peak 3 or 4 days after PMA treatment, which is relatively slower than the period required for other phorbol ester-responsive genes. These results suggest that the effect of phorbol ester on the MSR gene may be indirectly mediated.

Exon Organization and Protein Domains—Fig. 5 shows the
Human Macrophage Scavenger Receptor Gene

FIG. 8. Genetic linkage map of the region around MSR locus on human chromosome 8. The most likely order and distances of the six loci including the MSR locus as determined by genetic linkage analysis of 667 subjects in 59 CEPH and Utah reference families are indicated. Cen indicates the centromere. The physical locations of LPL (8p22), NFL (8p21), and the MSR locus are shown beside an idogram.

relationship between exon organization and protein domains as determined on the basis of protein sequence and in vitro mutagenesis experiments of the receptor expressed in cultured cells (2-4, 27). The introns interrupt the protein coding sequence in such a way that many of the protein segments are revealed as products of individual exons. Exon 1 encodes the 5'-untranslated region followed by a 12-kb intron which separates the transcription initiation site and the translation initiation site. The MSR gene has a collagen-like domain, and in the case of mouse α2(I) collagen gene, the enhancer element is located in the first intron, while in the case of the α1(I) collagen gene, insertional mutagenesis in the first intron resulted in a block of transcription of this gene (25, 26). The role of the first intron in MSR gene expression remains an open question. Exon 2 encodes the last 4 bases of the 5'-non-coding region and 70% of the cytoplasmic domain. Exon 3 encodes the remaining cytoplasmic domain and most of the transmembrane domain.

Exons 4 and 5 encode the region which possesses a cluster of possible N-linked sugar attachment sites. Analysis of the bovine and human receptor protein indicates that most of the potential sites are actually glycosylated (2, 11). This region includes an α-helical coiled-coil structure and spacer domain connecting a membrane-spanning domain and fibrous structure. Within the α-helical coiled-coil structure (2, 4), there are as many as 23 seven amino acid “heptad” repeats. Fig. 6 shows the comparison of predicted heptad repeats from various animal species. The repeats are divided into two groups due to the disruption of repeats by a skip at 204–211. The junction of exon 4 and 5 exactly matches this skip position, indicating that the α-helical coiled-coil domain of MSR consists of two coil structures encoded in different exons and that the junction generates a distortion of the coiled-coil which might be important for its function. The interruptions of hydrophobic amino acid repeats by histidines are encoded by both exons. These structural features are well conserved in the animal species studied. Analysis of the murine MSR gene confirmed that the position of the gap in the coiled-coil structure exactly corresponds to the position of the junction of the two exons.

Exons 6–8 encode the collagen-like structure. Exons 6 and 7 have a size of 81 bp encoding nine Gly-Xaa-Yaa triplets. In the case of fibrillar collagen genes, most exons in the triple helical domain have a size of 54, 45, 99, or multiples of 9 bp (26); this rule is conserved in exons of the MSR gene. Exon 8 encodes the five Gly-Xaa-Yaa triplets and the short C-terminal non-triple-helical region. In vitro mutagenesis experiments indicated that the cluster of basic amino acids encoded by exon 8 is essential for ligand binding.

Exon 9 encodes the type II-specific coding sequence and the 3'-non-coding sequence. Exons 10 and 11 encode the type I-specific coding sequence, the scavenger receptor cysteine-rich domain (SRCR domain), and the 3'-non-coding sequences. A group of genes which encode the domain highly homologous to the SRCR domain has been reported (27). The SRCR domain is divided into two regions. The N-terminal half contains 2 conserved cysteines, and a higher degree of identity among the amino acids of the SRCR proteins is present in this region. The C-terminal region of the SRCR domain has 4 cysteines; each cysteine residue appears every 10 amino acids. The N-terminal half is encoded by exon 10 and the C-terminal half by exon 11. The genes encoding proteins homologous to the SRCR domain (27) can be found in species from sea urchin (sea urchin speract receptor) to humans (CD5 and complement factor 1), suggesting that this domain may be used for the construction of complex mosaic proteins and/or mediates certain particular physiological functions.

As an integral membrane protein, MSR belongs to the so-called “inside-out” type receptor (2, 4). MSR lacks a signal sequence and have an N-terminal cytoplasmic domain, a single transmembrane domain and two different C-terminal extracellular domains. Alternative 3' splicing resulting in the generation of various proteins have been reported in several genes. In the case of the immunoglobulin constant region, secreted and membrane-bound forms are generated. In the case of the calcitonin gene, calcitonin and calcitonin gene-related proteins are generated by 3' alternative splicing, which is also related to cell-specific splicing activity. In the case of MSR, organization of the MSR gene is suitable for the generation of multiple C-terminal extracellular structures.

Physical and Genetic Mapping—We localized the human MSR gene cytogenetically on chromosomal bands by means of non-isotopic fluorescent in situ hybridization. High resolution mapping was facilitated by simultaneous staining of replicated prometaphase R-bands with propidium iodide (16). Fig. 7 demonstrates the result obtained with cosmide clone MSR35, which indicated that the MSR gene is located on human chromosomal band 8p22.

RFLP markers were then sought at the human MSR locus to carry out genetic linkage mapping. MSR cosmids DNAs were used as probes in Southern blot analysis of genomic DNA from six unrelated individuals; the DNA was digested with various restriction enzymes. Three common RFLPs were identified (Table II). A MspI RFLP with four alleles of 6.3, 3.1, 2.9, and 2.7 kb was detected by a 0.9-kb EcoRI-HindIII fragment of cMSR-32 as a probe. The same probe detected a BamHI RFLP with alleles of 14 and 9 kb. A HindIII RFLP with alleles of 13 and 9 kb was detected by a 9-kb HindIII fragment of cMSR-35. Allele frequency and heterozygosity, calculated from the data on 100 unrelated individuals of the reference families, are shown in Table II.

To locate MSR on the genetic map, genotypes of the three

2 T. Doi and T. Kodama, personal communication.
MSR RFLP systems were determined for 667 individuals in 59 CEPH and Utah reference families (28). To improve the information content of these systems, we used a MSR haplotype constructed by combining genotypes of all three RFLP systems, which gave a heterozygosity of 0.79. Pairwise linkage analyses of MSR haplotype showed significant linkage with five markers, D8S21, NEFL, D8S5, D8S17, and LPL. A sex-averaged linkage map of the six loci including the MSR was constructed by a multipoint linkage analysis with LINKAGE and GMS programs and the precise location of MSR in the linkage group was sought. This analysis established the most likely order and distances of the six loci as Tel.-D8S17-MSR-D8S21-LPL-D8S5-NEFL-Cen. and placed MSR locus 11 cM distal to LPL and 22 cM distal to NEFL, respectively (Fig. 8). LPL and NEFL have been localized cytogenetically to 8p22 and 8p21, respectively (29, 30). Therefore, highly significant linkage of the MSR locus with LPL and NEFL supports the cytogenetic assignment of MSR at 8p22.

MSR are thought to play an essential role in the metabolism of modified plasma lipoproteins by macrophages and are implicated in the pathogenesis of atherosclerosis. Genetic variations at the MSR locus may influence the susceptibility to atherosclerotic disorders. The RFLP markers identified in this study will enable future genetic linkage and association studies of those pathological conditions in which the MSR gene is a candidate gene. High heterozygosity (0.79), obtained by using the three systems jointly, will prove particularly useful in such investigations. Although the involvement of MSR in atherogenesis is well known, the physiological role of MSR remains obscure. The genetic study of the MSR gene may provide us further information concerning the physiological and pathological role of MSR.

Acknowledgments—We thank Dr. Monty Krieger of the Massachusetts Institute of Technology and Dr. Takefumi Doi of Osaka University for their helpful suggestions.

REFERENCES
1. Brown, M. S., and Goldstein, J. L. (1983) Annu. Rev. Biochem. 52, 223-261
2. Kodama, T., Freeman, M., Rohrer, L., Zabrecky, J., Matsuda, P., and Krieger, M. (1990) Nature 343, 531-535
3. Rohrer, L., Freeman, M., Kodama, T., Pennman, M., and Krieger, M. (1990) Nature 343, 570-577
4. Matsuzaki, M., Naito, M., Itakura, H., Ikemoto, S., Asaoka, H., Hayakawa, I., Kanamori, H., Aherutani, H., Takaku, F., Suzuki, H., Kobaru, Y., Miyazaki, T., Tashihashi, K., Cohen, E. H., Wydro, R., Housman, D. E., and Kodama, T. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 9133-9137
5. Hampton, R. Y., Golenbock, D. T., Pennman, M., Krieger, M., and Rasetz, C. R. (1991) Nature 353, 342-344
6. Freeman, M., Ekkeli, Y., Rohrer, L., Pennman, M., Freedman, N. J., Chisolm, G. M., and Krieger, M. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 4951-4955
7. Pennman, M., Lux, A., Freedman, N. J., Rohrer, L., Ekkeli, Y., McKinstry, H., Resnick, D., and Krieger, M. (1991) J. Biol. Chem. 266, 23893-23995
8. Naito, M., Kodama, T., Matsuzaki, M., Doi, T., and Tashihashi, K. (1991) Am. J. Pathol. 139, 1411-1423
9. Naito, M., Suzuki, H., Morii, T., Matsuzaki, M., Kodama, T., and Tashihashi, K. (1992) Am. J. Pathol. 141, 591-599
10. Sudhof, T. C., Goldstein, J. L., Brown, M. S., and Russell, D. W. (1985) Science 226, 813-822
11. Kodama, T., Reddy, P., Koshimoto, C., and Krieger, M. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 9236-9241
12. Frohman, M. A., Duax, M. K., and Martin, G. R. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 8986-9002
13. Yanakawa, K., Morita, R., Tashihashi, K., Hori, T., Lathrop, M., and Nakamura, Y. (1991) Genomics 11, 566-572
14. McKnight, S. L., and Kingsbury, R. (1982) Science 217, 316-324
15. Melton, D. A., Krieg, F. A., Rebagliati, M. R., Mannatis, T., Zinn, K., and Green, M. R. (1984) Nucleic Acids Res. 12, 7035-7056
16. Takahashi, E., Hori, T., O'Connell, F., Leppert, M., and White, R. (1990). Hum. Genet. 86, 14-19
17. Lathrop, G. M., Lalouel, J.-M., Julier, C., and Ott, J. (1985) Am. J. Hum. Genet. 37, 482-496
18. Lathrop, G. M., Nakamura, Y., Cartwright, P., O'Connell, F., Leppert, M., Jones, C., Tateishi, H., Bragg, T., Lalouel, J.-M., and White, R. (1988) Genomics 2, 107-104
19. Mount, S. M. (1982) Nucleic Acids Res. 10, 469-464
20. Proudfoot, N. J., and Whiteley, E. (1988) in Transcription and Splicing (Hames, B. D., and Glover, D. M. eds) pp. 57-129. IRL Press, Oxford
21. Shenk, T. (1981) Curr. Topics Microbiol. Immunol. 93, 25-38
22. Sudhof, T. C., Russell, D. W., Brown, M. S., and Goldstein, J. L. (1987) Cell 48, 1061-1069
23. Scolnick, E., Berthelot, R., Pfuffer, I., Schenk, B., Zarius, S., Swoboda, R., Murtzfer, F., and Karin, M. (1989) EMBO J. 8, 465-473
24. Angel, P., Imagawa, M., Chiu, R., Chiu, R., Stein, B., Imra, J. R., Brahmand, H. J., Jonat, C., Herlich, F., and Karin, M. (1987) Cell 48, 719-729
25. Harbans, K., Kuehn, M., Delius, H., and Jaenisch, R. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 1504-1508
26. Vuorio, E., and Crombrugge, B. D. (1990) Annu. Rev. Biochem. 59, 837-872
27. Freeman, M., Ashkenas, J., Rees, D. J., Kingsley, D. M., Copeland, N. G., Jenkins, N. A., and Krieger, M. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 8810-8814
28. White, R. L., Lalouel, J. M., Nakamura, Y., Donz-Keller, H., Green, F., Roschen, D. W., Mathew, C. G., Easton, D. Y., Robson, E. B., and Morton, N. E. (1990) Genomics 6, 305-412
29. Spanks, R. R., Zollman, S., Klisak, I., Kirchgersser, T. G., Komaromy, M. C., Mohandas, T., Suarez, M. C., and Lusis, A. J. (1990) Genomics 1, 138-144
30. Hurrel, J., Flavell, D., Julien, J.-P., Meijer, D., Mushynski, W., and Grosveld, F. (1987) Cytogenet. Cell Genet. 45, 30-32
31. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 5463-5467