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# Mapping of Human Methylmalonyl CoA Mutase (MUT) Locus on Chromosome 6

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## Summary

**Methylmalonyl CoA mutase (MCM) catalyzes an essential step in the degradation of several branch-chain amino acids and odd-chain fatty acids. Deficiency of this apoenzyme causes the *mut* form of methylmalonic acidemia, an often fatal disorder of organic acid metabolism. An MCM cDNA has recently been obtained from human liver cDNA libraries. This clone has been used as a probe to determine the chromosomal location of the MCM gene and MUT locus. Southern blot analysis of DNA from human-hamster somatic-cell hybrid cell lines assigned the locus to region q12-p23 of chromosome 6. In situ hybridization further localized the locus to the region 6p12-21.2. A highly informative RFLP was identified at the MCM gene locus which will be useful for genetic diagnostic and linkage studies.**

## Introduction

Methylmalonyl CoA mutase (MCM; E.C.5.4.99.2) catalyzes the isomerization of methylmalonyl CoA to succinyl CoA. This is an intermediate step in the degradation of valine, isoleucine, threonine, and methionine, odd-chain fatty acids, cholesterol, and thymine (reviewed in Rosenberg 1983). Deficiency of this enzyme causes methylmalonic acidemia (MMA), an often fatal disorder of organic acid metabolism in which the precursors and abnormal metabolites of methylmalonic acid accumulate, leading to widespread disruptions of metabolic homeostasis (Matsui et al. 1983; Rosenberg 1983). MCM is a homodimer with identical subunits of 72–77 $\times$ $10^3$ daltons (Kolhouse et al. 1980; Fenton et al. 1982) that requires adenosyl-cobalamin as a cofactor (Kolhouse and Allen 1977; Mellman et al. 1977). It is a mitochondrial protein, encoded by a nuclear gene, that is synthesized in the cytoplasm and must be transported into the mitochondria to express biological activity (Fenton et al. 1984, 1987).

Two classes of MMA are distinguished: defects in pathways of cobalamin metabolism designated *cbl* and defects in the MCM apoenzyme designated *mut* (reviewed in Rosenberg 1983). Somatic complementation studies demonstrate that fusion of cells from individuals with *mut* defects with those from individuals with *cbl* defects reconstitutes function of the MCM holoenzyme (Gravel et al. 1975), indicating that the *mut* and *cbl* phenotypes arise from mutations of distinct gene products (Willard et al. 1978). While different *mut* phenotypes can be distinguished clinically and biochemically (Willard and Rosenberg 1980; Matsui et al. 1983; Ledley et al. 1984), all are thought to constitute a single complementation group and thus to arise from allelic mutations at a single locus. At least six different *cbl* complementation groups have been distinguished that presumably represent distinct genetic loci (Rosenberg 1983; Watkins and Rosenblatt 1986).

The cloning of a cDNA for human MCM has recently been described elsewhere (Ledley et al., in press). Antibody against human placental MCM was

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used to screen a human liver cDNA library in λgt11. The authenticity of the MCM clone was established by gene transfer of the recombinant clone into cultured cells. Cells transformed with the recombinant clone were found to have three- to fivefold higher levels of MCM enzymatic activity than did controls. In addition, several cell lines with *mut* defects were found to have decreased or absent MCM mRNA (Ledley et al., in press).

In the present report the MCM cDNA has been used to establish the chromosomal location of the MCM gene and MUT locus. The MCM gene is assigned to chromosome 6p12-21.2 by mapping of somatic-cell hybrid cell lines and in situ hybridization.

## Material and Methods

### *MCM cDNA Probes*

Clone MCM26 is a cDNA in the vector λgt11 (Ledley et al., in press). This clone contains three internal *Eco*RI fragments designated *a–c*. The 5′-most fragment, MCM26*a*, contains 280-bp. The largest fragment, MCM26*b*, contains ~1,640 bp constituting the central region of the cDNA and the 3′ portion of the open reading frame (Ledley et al., in press). DNA fragments were extracted from low-melt agarose gels (Geneclean™) and nick-translated to a specific activity of $1 \times 10^9$ cpm/μg with [$^{32}$P] (Amersham) for Southern analysis.

### *Analysis of Human-Hamster Hybrids*

The construction and characterization of a panel of human–Chinese hamster somatic-cell hybrids for chromosome assignment has been reported elsewhere (Su et al. 1984; Chen et al. 1986). Human chromosomes were scored by analysis of at least 20 trypsin G-banded cells, followed by G-11 staining of selected cells (Su et al. 1984). Chromosomal studies and DNA extractions were done at the same passage.

A panel of somatic-cell hybrids containing fragments of chromosome 6 has been described elsewhere (Naylor et al. 1983). This work made use of cell lines RAG SU3-1-2-3, ITA9-1-2, GM610, RAG-4-5-1, and A9-1-13, provided by Dr. Karl Heinz Grzeschik.

DNA from somatic-cell hybrids were digested with *Eco*RI, *Bam*HI, or *Hin*dIII and analyzed by Southern blotting using methods described elsewhere (Lidsky et al. 1985; Ledley et al. 1987). Hybridization conditions (45% formamide, 4 × SSC, 0.1 M $NaPO_4$, 1 × Denhardt's, 0.1% Na-pyrophosphate, 0.1% SDS, 100 μg herring-sperm DNA/ml, and 10% dextran sulfate at 42 C) and ultimate wash conditions (0.2 × SSC, 0.5% SDS at 68 C) enable visualization of hamster, human, and mouse genomic restriction fragments.

### *In Situ Hybridization*

In situ hybridization was performed using the MCM26*b* fragment subcloned into pGEM4 (Promega) and $^3$H-labeled to a specific activity of $1 \times 10^8$ cpm/μg. Hybridization to metaphase chromosomes was carried out according to the method of Harper and Saunders (1981). Slides were dipped in Kodak NTB2 emulsion and exposed at 4 C for 14–21 days. After development, slides were banded by means of the sodium borate method of Cannizzaro and Emanuel (1984).

### *Identification of RFLPs*

Genomic DNA from six normal Caucasian subjects was digested with the enzymes *Alu*I, *Apa*I, *Bam*HI, *Ban*I, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*EII, *Bst*NI, *Dra*I, *Eco*RI, *Eco*RV, *Hinc*II, *Hin*dIII, *Hinf*I, *Kpn*I, *Mbo*I, *Msp*I, *Pss*I, *Pst*I, *Pvu*II, *Rsa*I, *Sac*I, *Sca*I, *Sph*I, *Sst*I, *Stu*I, or *Xba*I and probed with the MCM26*a* or MCM26*b* fragments. The allelic frequency of the *Hin*dIII polymorphism was calculated on the basis of analysis of DNA from 34 unrelated Caucasian individuals, including 12 normal individuals selected randomly and 22 individuals who are known to be carriers for phenylketonuria.

## Results

### *Identification of a Single MCM Gene Locus on Chromosome 6*

Southern hybridization of human genomic DNA cut with various enzymes to the MCM26*b* probe reveals a complex pattern with multiple restriction fragments. To determine the chromosomal location of this gene a panel of DNA from human-hamster or human-mouse somatic-cell hybrid cell lines cut with *Eco*RI (fig. 1*A*) or *Bam*HI (fig. 1*B*) was probed with the MCM26*b* probe (fig. 1). Restriction fragments representing the human, hamster, and mouse loci were easily distinguished on the basis of length. There was uniform concordance between the presence of restriction fragments representing the human MCM gene and the presence of human chromosome 6 in the somatic-hybrid cell line (fig. 2). All chromosomes were represented in at least five different cell lines in

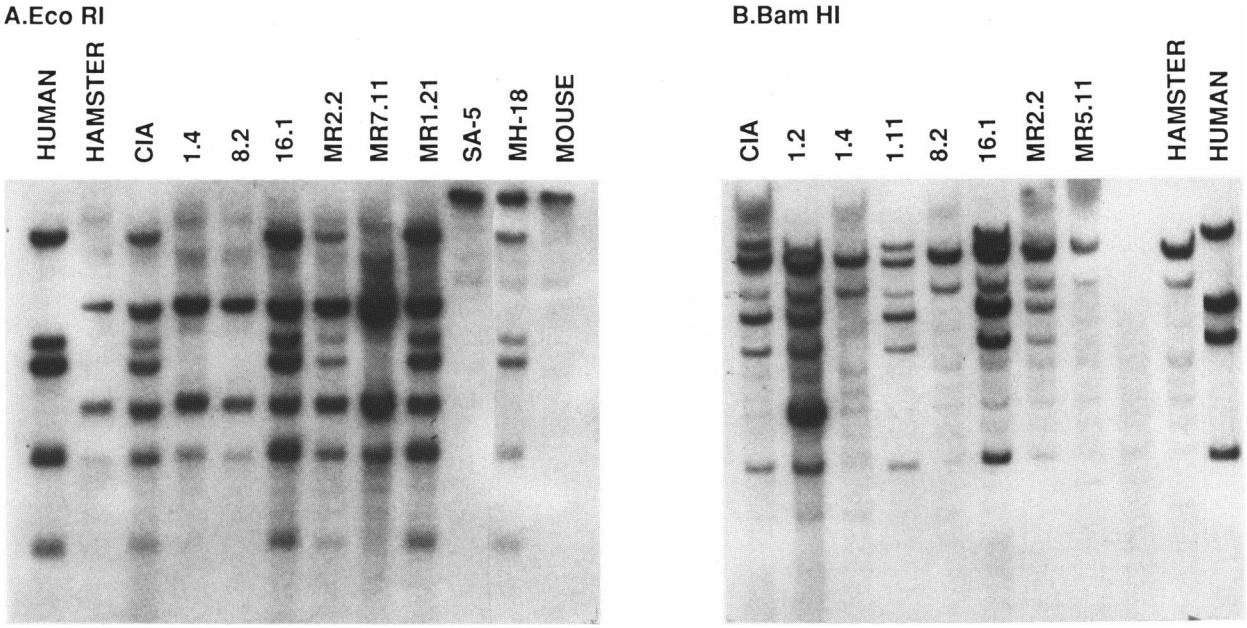


**Figure 1** Assignment of human MCM locus to chromosome 6. Shown is Southern blot analysis of panels with human-hamster and human-mouse hybrid cell lines probed with MCM26*b*. *A*, DNA digested with *Eco*RI. *B*, DNA digested with *Bam*HI. Restriction fragments for normal human, hamster, and mouse are indicated. Cell lines CIA, 1.2, 1.4, 1.11, 8.2, 16.1, MR2.2, MR5.11, MR7.11, and MR1.21 are human-hamster hybrids, and cell lines SA-5 and MH-18 are human-mouse hybrids (Su et al. 1984; Chen et al. 1986). The position of molecular-weight standards (in kb) is shown. The human chromosome complement of each cell line is shown in fig. 2. (An extraneous band in 1.2 that is not in control human or hamster DNA represents plasmid contamination.)

| CLONE | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | X | Y | HUMAN MCM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CIA | | | + | + | + | + | | | | | + | + | | + | + | + | + | + | | | + | + | + | | * |
| 1.2 | + | | + | + | + | + | + | + | + | | + | | | | | | | | + | + | + | | + | | * |
| 1.4 | | | + | + | | | | + | | | | | | | + | + | | | + | | + | | + | | |
| 1.11 | | | | | | + | | + | | | + | | | | | | | | | | | | + | | * |
| 8.2 | | + | + | + | | | + | + | + | + | + | + | | + | + | + | | | | + | | | + | | |
| 16.1 | | + | + | + | + | + | | | | | + | | | | | + | | + | + | | + | | + | + | * |
| MR 2.2 | | | + | + | | + | + | | | + | + | | + | | | | | | | + | | + | + | | * |
| MR 5.11 | + | + | + | | | | + | | + | + | + | | + | | | + | | + | | | + | + | + | | |
| MR 7.11 | | | | | | | + | | | | + | | + | | | | | + | | | | + | + | | |
| MR 1.21 | | | | | | + | + | | | | | + | | + | | | | | | | | | + | | * |
| SA-5 | | | | | | | + | | | | | + | | | +q | | +q | | | + | | | | | |
| MH-18 | | | + | | + | + | + | + | +q | | + | + | + | + | + | | +q | | | + | + | | | | * |
| | 42 | 33 | 58 | 58 | 75 | 100 | 42 | 50 | 42 | 33 | 66 | 50 | 42 | 66 | 33 | 33 | 50 | 42 | 50 | 50 | 58 | 42 | 58 | 50 | % CONCORDANCE |

**Figure 2** Concordance of human MCM hybridizing material with the presence of human chromosome 6 in human-hamster or human-mouse hybrid cell lines. The presence of human chromosomes in the hybrid cell lines illustrated in fig. 1 is indicated by a plus sign (+). Presence of the long arm without the short arm of a chromosome is indicated by "+q." The presence of restriction fragments corresponding to human MCM is indicated by an asterisk (*). Percent concordance between the presence of human MCM restriction fragments and the presence of human chromosome 6 for the 12 hybrid cell lines is indicated.

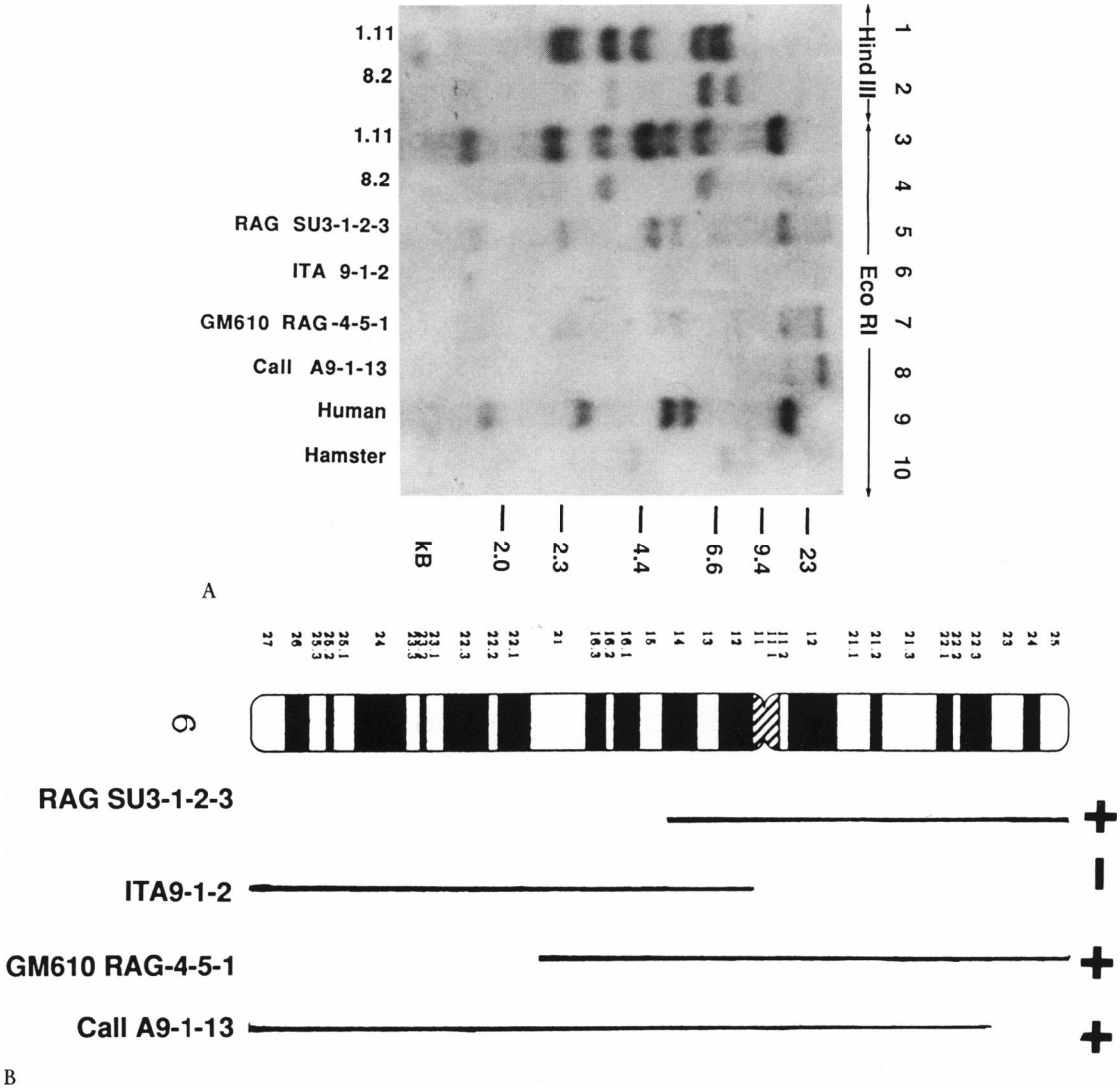


**Figure 3** Regional localization of human MCM locus using somatic cell hybrids. *A*, Southern blot of DNA from somatic-cell hybrids containing fragments of chromosome 6 (Naylor et al. 1983) probed with MCM26*b*. Lane 1, Human-hamster hybrid cell line 1.11 containing human chromosomes 6, 8, 11, and X digested with *Hin*dIII; lane 2, human-hamster cell line 8.2, which contains 8, 11, and X but not chromosome 6 digested with *Hin*dIII; lane 3, cell line 1.11 digested with *Eco*RI; lane 4, cell line 8.2 digested with *Eco*RI; lane 5, RAG SU3-1-2-3 digested with *Eco*RI; lane 6, ITA9-1-2 digested with *Eco*RI; lane 7, GM610 RAG-4-5-1 digested with *Eco*RI; lane 8, Call A9-1-13 digested with *Eco*RI; lane 9, human DNA digested with *Eco*RI; and lane 10, hamster DNA digested with *Eco*RI. The position of molecular-weight standards (in kb) is shown. *B*, Ideogram showing localization of human MCM in somatic cell hybrids. The regions of human chromosome 6 contained in the RAG SU3-1-2-3, ITA9-1-2, GM610 RAG-4-5-1, and A9-1-13 cell lines (Naylor et al. 1983) is indicated along with the presence (+) or absence (−) of human restriction fragments, as illustrated in *A*.

this panel; thus there are redundant data assigning the MCM gene locus to chromosome 6 and excluding all other chromosomes. This result was confirmed by the demonstration that human restriction fragments were present in cell line 1.11, which contains human chromosomes 6, 8, 11, and X (fig. 3, lanes 1, 3), but not in cell line 8.2, which contains human chromosomes 8, 11, and X (and others) but not 6 (fig. 3, lanes 2, 4). Significantly, each of the *Bam*HI, *Hin*dIII, and *Eco*RI restriction fragments visualized with the MCM26*b* probe in human genomic DNA can be independently assigned to chromosome 6 on the basis of analysis of the data represented in figure 1 or in figure 3.

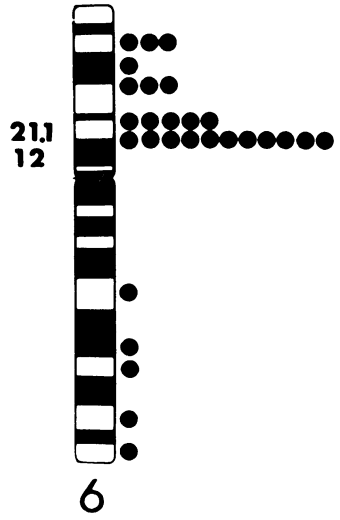


**Figure 4** In situ hybridization of human chromosomes with MCM26*b* probe. Ideogram showing position of 28 grains counted overlying chromosome 6. Sixteen of 28 (57%) of all grains over chromosome 6 are in a single peak at 6p12-21.2

### *Regional Localization of the MCM Gene Locus on Chromosome 6*

To further localize the MCM gene locus, a panel of DNA from cell lines containing fragments of chromosome 6 (Naylor et al. 1983) was probed with the MCM26*b* probe (fig. 3, lanes 5–8). Restriction fragments of the human MCM locus were identified in cell line RAG SU3-1-2-3 containing 6pter-q14 (fig. 3, lane 5), in GM610 RAG-4-5-1 containing 6pter-q21 (fig. 3, lane 7), and in Call A9-1-1-13 containing 6-23-qter (fig. 3, lane 8) but not in cell line ITA9-1-2 containing 6q12-qter (fig. 3, lane 9). These analyses localized the region of the MCM gene locus to 6p23-q12 (fig. 3*B*).

The MCM locus was further localized by in situ hybridization. Of 200 grains observed, 28 (14%) were on chromosome 6. The distribution of 28 grains localized on chromosome 6 is shown in figure 4. Sixteen (58%) of these 28 were in the region 6p12-21.2, a result confirming and refining the localization made on the basis of somatic-cell hybrids.

### *Identification of RFLPs*

DNA from six unrelated individuals was screened with 28 different enzymes for the presence of RFLPs by using the MCM26*a* and MCM26*b* probes. A *Hin*dIII polymorphism was identified using the MCM26*b* probe in six of 12 alleles of this initial panel (fig. 5*A*) and in five of 12 alleles from a subsequent panel of six additional individuals. The polymorphism was apparent in either a 7-kb *Hin*dIII(−) allele or a 3-kb *Hin*dIII(+) allele. When the first filter was reprobed with MCM26*a*, a complementary polymorphism was observed (fig. 5*B*). The 7-kb fragment observed with the MCM26*a* probe on *Hin*dIII(−) alleles appears to be identical to that ob-

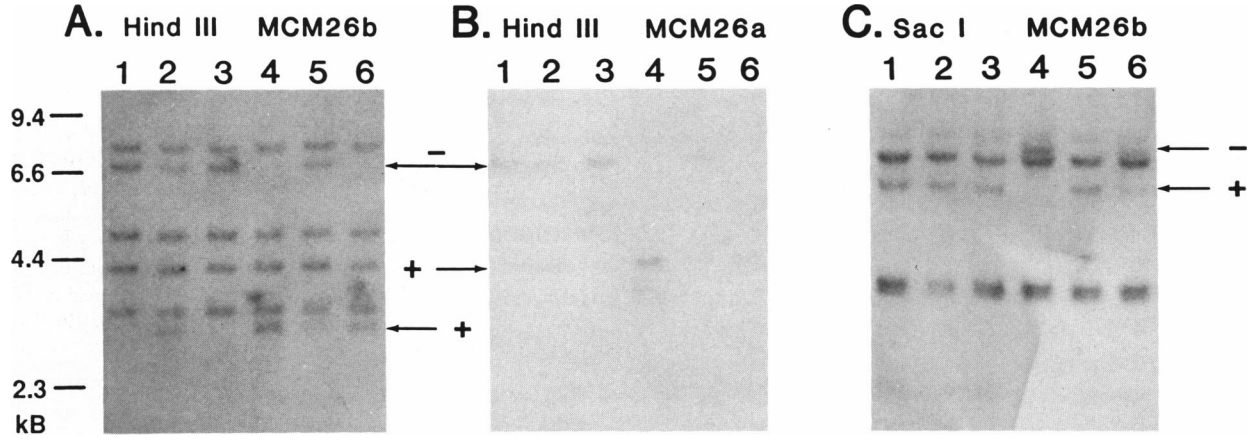


**Figure 5** Polymorphisms at the MCM locus. DNA from six unrelated Caucasians was digested with the indicated restriction enzyme and probed with MCM26*b* or MCM26*a*. Polymorphic bands are indicated by arrows. Bands resulting from the absence of a polymorphic site are designated by a minus sign (−); those resulting from the presence of a polymorphic site are designated by a plus sign (+). *A*, Genomic DNA digested with *Hin*dIII and probed with MCM26*b*; *B*, genomic DNA digested with *Hin*dIII (same filter as in *A*) and probed with MCM26*a*; *C*, genomic DNA digested with *Sac*I and probed with MCM26*b*. The characteristic pattern and distribution of the *Hin*dIII polymorphisms identified with the MCM26*a* and MCM26*b* probes suggest that there is a single *Hin*dIII polymorphic site that is flanked by the two hybridizing probes. The position of molecular-weight standards (in kb) is shown.

served with the MCM26*b* probe, and a distinct 4-kb band is observed on the *Hin*dIII(+) allele (fig. 5*B*). The coincidence of the polymorphisms identified with the MCM26*a* and MCM26*b* probes among 12 alleles examined, and the fact that the size of the two bands observed on the *Hin*dIII(+) allele (3 kb + 4 kb) together approximate the size of the common band observed on the *Hin*dIII(−) allele (7 kb), suggests that the MCM26*a* and MCM26*b* probes flank a single *Hin*dIII polymorphic site.

A panel containing DNA from 11 families (44 alleles) was screened with the MCM26*b* probe, and in each case the *Hin*dIII polymorphism segregated in a Mendelian codominant manner (data not shown).

A total of 68 alleles were screened for the *Hin*dIII polymorphism. Of 68 alleles, 31 contained the *Hin*dIII(+) polymorphic site (46%). Of 34 individuals, seven (20%) were homozygous for the *Hin*dIII(−) allele, 10 (30%) were homozygous for the *Hin*dIII(−) allele, and 17 (50%) were heterozygous.

A single individual with an apparent *Sac*I polymorphism (fig. 5*C*) was identified (one of 12 alleles) but was not characterized further.

## Discussion

This work identified the human MCM gene locus on chromosome 6p12–21.2 by using several different techniques. The initial chromosomal and regional assignment was made by Southern blot hybridization of panels of human-hamster and human-mouse hybrid cell lines with the MCM gene probe. These experiments demonstrated complete concordance between the presence of the human MCM gene locus and human chromosomal fragments spanning 6p23–q12. Results of in situ hybridization analysis were consistent with this assignment and enabled further localization of the MCM gene locus to 6p12–21.2.

Southern blotting of human genomic DNA with the MCM26*b* probe reveals multiple hybridizing bands. It is important that each of the restriction fragments visualized with the MCM26*b* probe using *Bam*HI, *Eco*RI, or *Hin*dIII independently maps to the same location in the panels of somatic-cell hybrid cell lines. This finding indicates that the multiple hybridizing bands observed in human genomic DNA constitute a single locus on chromosome 6 rather than a dispersed family of homologous genes or pseudogenes at multiple loci. The possibility that there are a series of homologous genes within the identified MCM gene locus at 6p12–21.2 cannot be unequivocally excluded on the basis of the present analysis. The fact that the MCM26*a* probe, which represents the 5′ end of the cDNA, recognizes only a single band in human genomic DNA when digested with most enzymes suggests that there is only a single-copy MCM gene that contains exons spanning ≥50 kb.

MMA can be caused by deficiency of the MCM apoenzyme (designated *mut*) or by defects in the availability, metabolism, or transport of cobalamin (vitamin B12) (designated *cbl*) (Gravel et al. 1975; Willard et al. 1978; Watkins and Rosenblatt 1986; reviewed in Rosenberg 1983). Classical somatic-cell complementation studies demonstrate that *mut* and *cbl* defects involve mutations at distinct genetic loci (Willard et al. 1978; Willard and Rosenberg 1980; reviewed in Rosenberg 1983). Various clinical forms of *mut* deficiency have been described elsewhere (Matsui et al. 1983; Ledley et al. 1984), and subsets of *mut* deficiency have been discriminated on the basis of the presence or absence of residual enzymatic activity or cross-reactive material (CRM) (Willard and Rosenberg 1980; Kolhouse et al. 1981). Nevertheless, *mut* deficiency represents a single complementation group, and the various *mut* defects are thought to represent allelic variation at a single locus, which, in humans, is designated MUT. Preliminary studies demonstrate that fibroblasts from some individuals with *mut* deficiency have absent or decreased mRNA hybridizable to the MCM26*b* clone (Ledley et al., in press). This indicates that the MCM26*b* clone recognizes the mRNA product of the MUT locus. Thus, the gene locus identified with the MCM26 probe at 6p12–21.2 is the MUT locus identified previously by somatic-cell complementation (Willard et al. 1978). This locus is distant from the locus for the other cobalamin-requiring enzyme (homocysteine–$N^5$–methylenetetrahydrofolate methyltransferase, at least one subunit of which has been provisionally localized to chromosome 1 (Mellman et al. 1979).

The present analysis identified only two polymorphisms at the restriction sites of 28 enzymes tested, despite the fact that multiple bands were identified with each enzyme. A minimum of 720 bases were examined by this analysis (calculated as [no. of bands +1] × recognition sequence length for each enzyme), giving a polymorphic frequency of <1/360 bases examined. Nevertheless, the *Hin*dIII polymor-

phism identified with both the MCM26*b* and MCM26*a* probes should be useful for genetic analysis of families with *mut* deficiency and for extending the linkage map of chromosome 6p. Since the MCM gene probe has been shown to recognize the gene product of the MUT locus, this RFLP may be useful for genetic diagnosis in families in which a proband is known to have *mut* deficiency.

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