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Dominant Inheritance of Isolated Hypermethioninemia Is Associated with a Mutation in the Human Methionine Adenosyltransferase 1A Gene

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Summary

Methionine adenosyltransferase (MAT) I/III deficiency, characterized by isolated persistent hypermethioninemia, is caused by mutations in the *MAT1A* gene encoding MATα1, the subunit of major hepatic enzymes MAT I ($[\alpha1]_4$) and III ($[\alpha1]_2$). We have characterized 10 *MAT1A* mutations in MAT I/III–deficient individuals and shown that the associated hypermethioninemic phenotype was inherited as an autosomal recessive trait. However, dominant inheritance of hypermethioninemia, also hypothesized to be caused by MAT I/III deficiency, has been reported in two families. Here we show that the only mutation uncovered in one of these families, G, is a G→A transition at nt 791 in exon VII of one *MAT1A* allele that converts an arginine at position 264 to a histidine (R264H). This single allelic R264H mutation was subsequently identified in two hypermethioninemic individuals in an additional family, C. Family C members were also found to inherit hypermethioninemia in a dominant fashion, and the available affected members analyzed carried the single allelic R264H mutation. Substitution of R-264 with histidine (R264H, the naturally occurring mutant), leucine (R264L), aspartic acid (R264D), or glutamic acid (R264E) greatly reduced MAT activity and severely impaired the ability of the MATα1 subunits to form homodimers essential for optimal catalytic activity. On the other hand, when lysine was substituted for R-264 (R264K), the mutant α1 subunit was able to form dimers that retain significant MAT activity, suggesting that amino acid 264 is involved in intersubunit salt-bridge formation. Cotransfection studies show that R264/R264H MATα1 heterodimers are enzymatically inactive, thus providing an explanation for the R264H-mediated dominant inheritance of hypermethioninemia.

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Introduction

Newborns are routinely screened for high levels of plasma methionine as an indicator of homocystinuria caused by cystathionine β-synthase deficiency (Mudd et al. 1995*a*). A subset of children with high plasma methionine has been found to have isolated persistent hypermethioninemia (Mudd et al. 1995*b*). Six such individuals were diagnosed by enzyme studies as having deficient methionine adenosyltransferase (MAT) activity in the liver but normal MAT activities in their cultured skin fibroblasts, red blood cells, or lymphoid cells (Finkelstein et al. 1975; Gaull et al. 1981; Gahl et al. 1987). There are two human MAT genes, *MAT1A* and *MAT2A* (Kotb et al., in press). *MAT1A* is a single-copy gene expressed primarily in the liver and encoding two isozymes, MAT I and III, that are tetramers and dimers formed from identical α1 subunits (Cabrero et al. 1987). MAT II, encoded by the *MAT2A* gene, is found in fetal liver (and, to a lesser extent, in adult liver) as well as in kidney, brain, testis, and lymphocytes (Kotb and Kredich 1985; Mitsui et al. 1988; Horikawa and Tsukada 1992; De La Rosa et al. 1995). Thus, the isolated persistent hypermethioninemic phenotype characterized by low liver MAT activity is associated with MAT I/III deficiency.

MAT catalyzes the biosynthesis of S-adenosylmethionine (AdoMet) from methionine and ATP (Cantoni et al. 1953). AdoMet participates in transmethylation and transsulfuration pathways and, after decarboxylation, serves as a propylamine group donor in the biosynthesis of polyamines (Kotb and Geller 1993; Mudd et al. 1995*a*). The majority of MAT I/III–deficient individuals have been without major clinical abnormalities (Mudd et al. 1995*b*). However, two individuals with isolated hypermethioninemia have developed neurological deficits, including brain demyelination, suggesting that MAT I/III deficiency can be deleterious (Surtees et al. 1991; Mudd et al. 1995*b*). To study the molecular genetics of MAT I/III deficiency, we characterized the human *MAT1A* gene and showed that it is a single-copy gene consisting of nine exons and eight introns (Ubagai et al. 1995). We also characterized the *MAT1A* gene in 11

individuals with isolated persistent hypermethioninemia, including 4 patients with confirmed MAT I/III deficiency and 2 with brain demyelination. A total of 10 mutations that abolish or reduce MAT activity were detected in the *MAT1A* gene of the 11 individuals, thus establishing the molecular basis of this disorder (Ubagai et al. 1995; Chamberlin et al. 1996). Our studies also showed that a complete lack of MAT I/III activity can lead to neurological abnormalities and brain demyelination (Chamberlin et al. 1996).

Mutational analysis revealed that the 11 MAT I/III–deficient individuals are either homozygous for defects in the *MAT1A* gene or compound heterozygotes (Ubagai et al. 1995; Chamberlin et al. 1996). Analysis of the *MAT1A* gene in the available parents of these patients indicated that the hypermethioninemia associated with these mutations behaves as an autosomal recessive trait (Ubagai et al. 1995; Chamberlin et al. 1996). There are, however, two recent reports of persistent hypermethioninemia inherited in a dominant fashion (Blom et al. 1992; Mudd et al. 1995*b*). Because enzymatically active MAT is a MATα1 oligomer (Cabrero et al. 1987), a mutation in the *MAT1A* gene causing a mutant subunit to adversely affect enzymatic activity of heterodimers formed with a wild-type MATα1 subunit might exert a dominant deleterious effect. We therefore characterized the *MAT1A* gene in members of two families with dominant inheritance of hypermethioninemia. All affected members genotyped from both families were found to contain, within only one *MAT1A* allele, a G→A mutation at nt 791 that converts an arginine at position 264 to a histidine (R264H). Our studies also suggest that amino acid 264 in MATα1 is involved in salt-bridge formation essential for subunit dimerization and that the dominant effect of the R264H mutation is exerted via the formation of enzymatically inactive R264/R264H dimers.

Subjects and Methods

Isolated Persistent Hypermethioninemic Individuals

Family G was described by Blom et al. (1992). Two hypermethioninemic members, CIII-1 and CIII-2, in family C were identified by routine screening of newborns (Mudd et al. 1995*b*). Plasma methionine levels of other family C members were determined in the present study. Genomic DNA preparations from patients were isolated from blood samples with a Nucleon II kit obtained from Scotlab. Peripheral blood samples were obtained with the informed consent of the individuals.

Analysis of the MAT1A *Gene*

The *MAT1A* gene was characterized by amplifying the coding regions of exons I–IX and the corresponding intron-exon junctions by PCR as described by Ubagai et al. (1995). The PCR-amplified fragments were analyzed for SSCP (Orita et al. 1989) by electrophoresing wild-type and mutant target DNAs side by side through mutation detection electrophoresis (MDE) nondenaturing gels (AT Biochem) containing 5% glycerol following the protocols provided by the manufacturer. Potential mutations in the target DNA were identified by the differential migration of one or both of the amplified strands. To confirm the presence of mutations, amplified fragments were subcloned into the pNoTA/T7 vector with the Prime PCR Cloning System (5′-3′, Inc.), and five or more subclones of each exon were sequenced.

The G791A/R264H mutation was identified also by allele specific oligonucleotide (ASO) hybridization using either a wild-type (5′-CACTGGCCGTAAGATTAT-3′) or a mutant (5′- CACTGGCCATAAGATT AT-3′) probe corresponding to nt 783–800 in the human *MAT1A* cDNA (Ubagai et al. 1995). In brief, the exon VII DNAs were applied onto a Nytran membrane (Schleicher and Schuell) with a slot-blot apparatus (Manifold II, Schleicher Schuell) and hybridized overnight at 42°C in a buffer containing 6× SSC, 4× Denhardt's, 1 mM EDTA, 0.1 mg/ml salmon sperm DNA, and an end-labeled oligonucleotide probe. Blots were washed in 6× SSC at 52°C for 5 min and autoradiographed.

Construction of Amino Acid 264 Mutants

We employed a modification of cassette mutagenesis (Wells et al. 1985) to generate multiple mutations of codon 264. The pSVLhMAT-*Apa*I-3′ fragment (containing nt 664–1188 of human *MAT1A*) was used for construction of codon 264 mutants by site-directed mutagenesis (Ubagai et al. 1995). The two outside PCR primers were nt 664–801 and nt 781–1188, and the sense strand of the degenerate inside primers (nt 781–801) was 5′-GTCACTGGC (C/A/G)(A/T)(T/A/C)AAGA TTATT-3′. The amplified fragments were digested with *Apa*I and *Xba*I and ligated into the pSVLhMAT-*Apa*I-5′ fragment as described by Ubagai et al. (1995). Mutants were identified by DNA sequencing. A *Bam*HI fragment containing the entire mutant cDNA was also subcloned into a bacterial expression vector, pQE30 (QIAexpress System, QIAGEN, Inc.).

Expression of Mutant MAT1A *cDNAs*

M15 bacteria were transformed with wild-type or mutant *MAT1A* cDNAs in the pQE30 vector, and COS-1 cells were transfected with wild-type or mutant *MAT1A* cDNAs in the pSVL vector as described by Chamberlin et al. (1996). MAT activity was assayed essentially as described by Mitsui et al. (1988). In brief, the enzyme was incubated for 30 min at 37°C in a reaction mixture (100 μl) containing 0.1 M Tris-HCl, pH 8.2, 20 mM $MgCl_2$, 150 mM KCl, 10 mM ATP, 5 mM β-mercaptoethanol, and 500 μM L-[methyl-^{3}H] methionine (0.5 μCi/reaction) or 500 μM L-[methyl-^{35}S] methionine (0.5

μCi/reaction). The reaction was stopped with 10 μl of 2 M $HClO_4$ containing 5 mM methionine. After centrifugation, 50 μl of the supernatant solution was spotted onto a phosphocellulose circle, washed in 5 mM methionine/1% casamino acids, dried, and measured in a scintillation counter.

Cross-Linking of MATα1 and Western Blot Analysis

Extracts (220 μg protein in 100 μl) from bacteria expressing a wild-type or mutant *MAT1A* cDNA were incubated with 2.6 μg of Bis (sulfosuccinimidyl)suberate (BS^3) (Pierce) at room temperature for 20 min. The reaction was stopped by the addition of 15 μl of 1M glycine, and the reaction products were electrophoresed through 10% SDS-polyacrylamide gels and transferred onto Immobilon P (Millipore). The MATα1 protein was detected using a rabbit anti-MATα1 serum (Chamberlin et al. 1996) and an alkaline phosphatase system (Kirkegaard and Perry Laboratories, Inc.).

Results

All Hypermethioninemic Members in Family G Carry a Single Allelic R264H Mutation in Their MAT1A *Genes*

Analysis of plasma methionine levels in family G (fig. 1) led Blom et al. (1992) to propose that a dominant mutation in the *MAT1A* gene might be responsible for the hypermethioninemic phenotype of this family. To test this hypothesis, we employed SSCP analysis to identify potential mutations in the nine exons of the *MAT1A* gene in three members of family G (GI-1, GI-2, and GII-1, fig. 1). SSCP variations were observed in exons III, VII, VIII, and IX fragments when compared with those of the wild-type gene. Sequence analysis showed that changes in exons III (C225T/A75A), VIII (C→T, intron 8), and IX (T1131C/Y377Y) were silent. The SSCP pattern of exon VII was complex (fig. 2*A*); however, all affected members analyzed shared one SSCP band (indicated by the arrows in fig. 2*A*). Sequence analysis of five or more exon VII subclones from GI-1, GI-2, and GII-1 revealed that the two affected members, GI-1 and GII-1, each had a G→A transition at nt 791 that converts an arginine at position 264 to a histidine (R264H) (fig. 3). Roughly half of their exon VII subclones contained this mutation, suggesting that only one *MAT1A* allele was affected. In contrast, all exon VII subclones of GI-2, who had normal plasma methionine levels, contained wild-type *MAT1A* sequence (G791).

The presence of the R264H mutation in exon VII of family G members was analyzed by ASO hybridization using oligonucleotide probes specific to a wild-type (G791) or a mutant (G791A) *MAT1A* allele (fig. 2*B*). Amplified exon VII DNA from all affected members hybridized to both probes, indicating that they have both wild-type (R264) and mutant (R264H) alleles (fig. 2*B*).

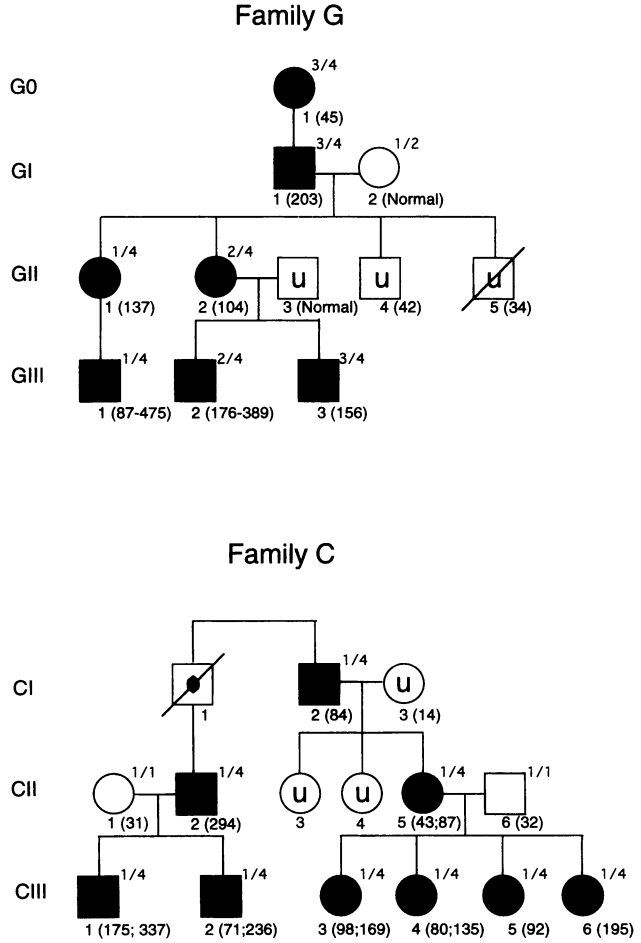

Figure 1 Pedigrees of families G and C with dominant inheritance of hypermethioninemia. The pedigree of family G and the members' plasma methionine levels were reported by Blom et al (1992), except for G0-1, who was analyzed in this study. The plasma methionine levels of CIII-1 and CIII-2 of family C were reported by Mudd et al (1995b). Black-filled symbols represent hypermethioninemic subjects carrying the single allelic R264H mutation, open symbols represent normal subjects, open symbols containing the letter u represent individuals not available for genotyping, and the open symbol with a filled dot represents an obligatory carrier. Values in parentheses are plasma methionine levels (in μM). The upper limit of reference human plasma methionine levels is 35–45 μM (Blom et al. 1992; Mudd et al. 1995b). Plasma methionine concentrations in GIII-1 and GIII-2 are ranges of three determinations and in CII-5 and CIII-1, -2, -3, and -4 are two individual measurements. For CII-5, the lower value was obtained when the subject was on progesterone. For CIII-1, -2, -3, and -4, the lower values were obtained when the subjects were ages 1.8–14 years; the higher values, ages 1 mo–1 year. The *MAT1A* exon VII haplotype of each genotyped member is denoted at the upper right corner of each symbol.

On the other hand, exon VII DNA of an unaffected family member, GI-2, like the DNA from a wild-type subject, hybridized only to the wild-type probe (fig. 2*B*). The presence of the R264H mutation in family G members was confirmed also by DNA sequencing (data not shown).

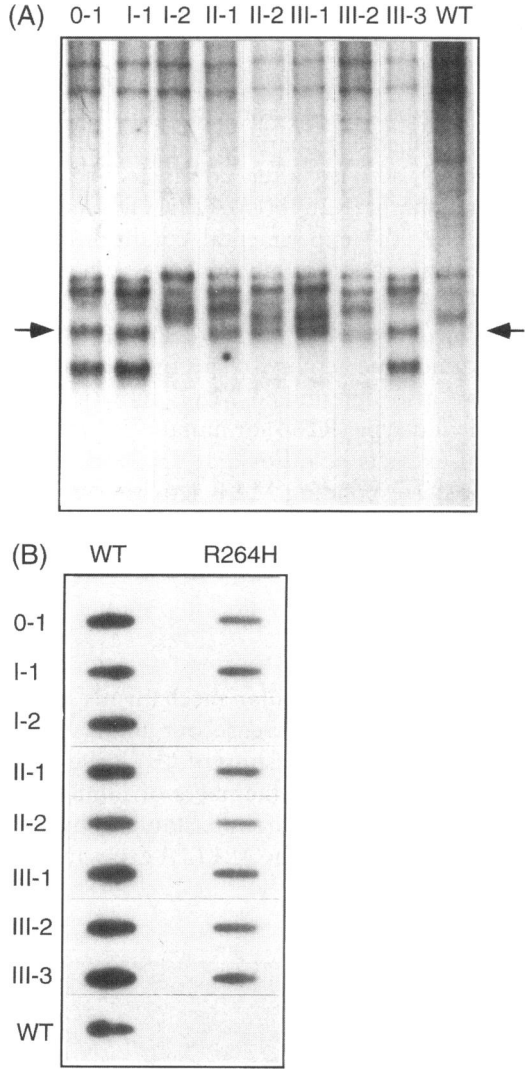

Figure 2 Molecular analysis of the *MAT1A* gene of family G members. *A*, SSCP analysis of PCR-amplified exon VII fragments on MDE gels containing 5% glycerol. The exon VII fragment of an unrelated, unaffected subject is denoted as wild-type (WT). The common SSCP band identified in all affected members is denoted by arrows. *B*, ASO hybridization analysis of PCR-amplified exon VII fragments affixed to a nytran membrane as described in Subjects and Methods.

Studies have shown that 5%–25% of mutations in PCR products escape detection by SSCP analysis (Orita et al. 1989). To search for mutations in the *MAT1A* gene of hypermethioninemic members in family G, in addition to R264H, we sequenced five subclones each from exons I–IX of patient GII-1 but detected no additional mutations (data not shown).

The complex SSCP pattern of *MAT1A* exon VII (fig. 2*A*) suggests the presence of sequence variations or additional mutations. Sequence analysis revealed the existence of four allelic variants in exon VII fragments of family G (table 1). Variants 1–3 encode the wild-type MATα1, and variant 4 (G791A) encodes the R264H mutant. The exon VII haplotypes of genotyped family G members are indicated in figure 1.

Table 1

Allelic Variants in *MATIA* Exon VII and the Associated Intron 7 of Families G and C

	Nucleotide			
Allele	791[a]	870[b]	882[b]	44-Intron 7
1 (Normal)	G	G	C	C
2 (Normal)	G	G	C	T
3 (Normal)	G	A	T	C
4 (Mutant)	A	G	C	C

[a] G-to-A mutation at nt 791 converts an arginine at residue 264 to a histidine.

[b] G-to-A transition at nt 870 and C-to-T transition at nt 882 result in no amino acid change.

The R264H-Associated Hypermethioninemic Phenotype Is Transmitted in a Dominant Fashion

Mutation analysis revealed the presence of this single allelic R264H mutation in the *MAT1A* gene in two additional hypermethioninemic individuals, CIII-1 and CIII-2 from family C (fig. 1). SSCP and sequencing

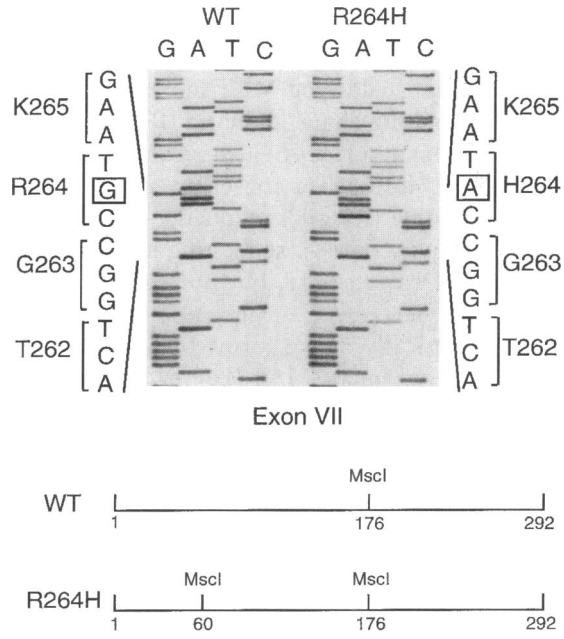

Figure 3 Autoradiogram of Sanger nucleotide sequence of the *MAT1A* gene in the hypermethioninemic patient GI-1. GI-1 contains a wild-type (G791/R264) and a mutant (G791A/R264H) *MAT1A* allele. The G→A (boxed) transition at nt 791 generates an additional *Msc*I site in exon VII that can be used as a diagnostic marker. Whereas *Msc*I digestion of the amplified wild-type (WT) exon VII fragment (292 bp) yielded two fragments of 176 and 116 bp, the R264H exon VII fragment yielded three fragments of 60, 116, and 116 bp.

analyses detected no additional mutations in the *MAT1A* gene of CIII-1 or CIII-2. Moreover, the mother of CIII-1 and CIII-2 (CII-1, fig. 1) had a normal serum methionine level of 31 μM and carried no mutations in her *MAT1A* gene.

The G→A transition at nt 791 generates an additional *Msc*I site in exon VII that can be used as a diagnostic marker (fig. 3). Plasma methionine levels and the appearance of a new *Msc*I restriction site in one allele of *MAT1A* exon VII were examined in family C members (fig. 1). Our results show that all hypermethioninemic individuals in family C available for genotyping also had one wild-type (G791/R264) and one mutant (G791A/R264H) *MAT1A* allele. Thus, the mild hypermethioninemic phenotype associated with the R264H mutation is also transmitted in a dominant fashion in family C. Sequence analysis revealed that the *MAT1A* gene of genotyped family C members contained exon VII allelic variants 1 and 4 (fig. 1).

Amino Acid 264 in MATα1 Is Involved in Subunit Dimerization

To examine the structural requirement of residue 264 in MAT catalysis, we constructed a series of codon 264 mutants in pQE30 and analyzed for MAT activity after expression in *Escherichia coli*. The R-264 was substituted with amino acids of diverse structures including histidine (R264H, the naturally occurring mutant), lysine (R264K), leucine (R64L), glutamic acid (R264E), and aspartic acid (R264D) (fig. 4). The R264H mutant had <1% of the wild-type MAT activity. Substitution of R-264 with long-chain aliphatic (R264L) or acidic residues (R264D and R264E) also greatly reduced enzymatic activity. However, a conservative substitution of lysine for arginine (R264K) retained ~20.4% of wild-type MAT activity (fig. 4), suggesting a positive charge at residue 264 is necessary, but not sufficient, for full enzymatic activity.

The enzymatically active forms of MAT are dimers (MAT III) and tetramers (MAT I) of the MATα1 subunit (Cabrero et al. 1987). The positive charge requirement for amino acid 264 in MATα1 suggests that this residue may be involved in salt-bridge formation essential for subunit dimerization. We therefore examined the ability of bacterially expressed wild-type and mutant MATα1 to dimerize by treating the bacterial extracts with a chemical cross-linker, BS^3. Western blot analysis showed that wild-type MATα1 subunits readily formed dimers (fig. 4). On the other hand, MATα1 mutants (R264H, R264L, R264D, and R264E), lacking a strong positive charge at position 264, failed to dimerize under the experimental conditions used (fig. 4). However, the R264K-MATα1 did dimerize, suggesting that a positively charged amino acid at this position is required for polar interactions during intersubunit dimerization.

The R264/R264H MATα1 Heterodimer Is Enzymatically Inactive

To exert a dominant effect on MAT enzymatic activity, the wild-type and mutant heterodimer should have greatly diminished enzymatic activity. We therefore examined MAT activity after cotransfecting wild-type (R264) and mutant (R264H) *MAT1A* cDNAs into COS-1 cells. Under the experimental conditions used, the R264H mutant was virtually inactive. When R264 to R264H cDNAs were transfected at ratios 3:1, 1:1, and 1:3, MAT activities observed were 41.9%, 28.8%, and 12.2% of wild-type activity, respectively (table 2). As a control, we also determined MAT activity by mixing individual wild-type (R264) or mutant (R264H) cDNA-transfected extracts at ratios 3:1, 1:1, and 1:3. As expected, the corresponding MAT activities were 73.9%, 54.1%, and 28.8%, respectively, of wild-type activity (table 2), reflecting the amount of wild-type extract present in the assays.

Discussion

To delineate the molecular mechanisms of a dominant mutation and to increase our understanding of structure-function relationships of MAT, we characterized the *MAT1A* gene in members of family G (Blom et al. 1992) with a dominant inheritance of hypermethioninemia. We show that the *MAT1A* genes in all hyper-

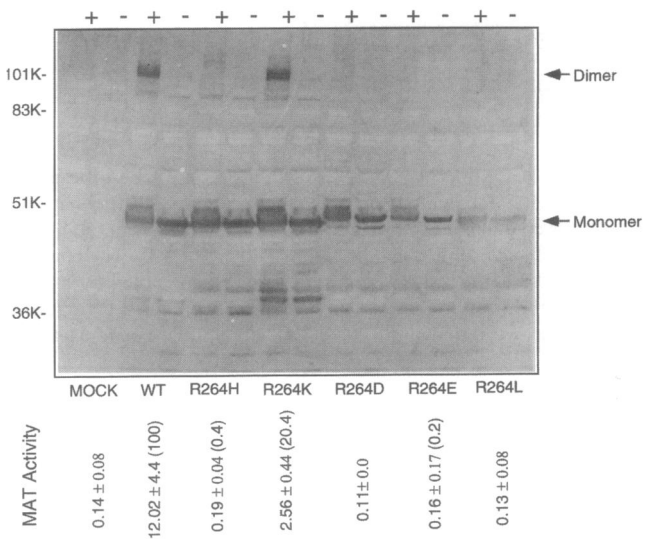

Figure 4 Enzymatic activity of bacterial extracts transformed with wild-type or mutant *MAT1A* cDNAs and Western blot analysis of cross-linked MATα1 subunits. Extracts (220 μg in 100 μl) were incubated in the absence (−) or presence (+) of a chemical cross-linker, BS^3, electrophoresed through a 10% SDS-polyacrylamide gel, blotted onto Immobilon P, and probed with an anti-MATα1 serum (Chamberlin et al. 1996). The apparent molecular weight of the monomers and dimers are 47 kD and 102 kD, respectively. The enzymatic activity (in nmole/min/mg protein) is depicted below each corresponding lane. Numbers in parentheses represent percent of total activity.

Table 2

MAT Activity from COS-1 Cell Extracts

Wild-Type/R264H (Ratio)	MAT Activity (% Wild Type)	
	Cotransfected	Mixed
3:1	41.9 ± 7.8	71.9
1:1	28.8 ± 8.2	54.1
1:3	12.2 ± 7.9	28.8

NOTE.—The MAT activities in nmol/min/mg protein are: wild-type (R264), .39 ± .4; R264H, .16 ± .2; and mock, .18 ± .2. Cotransfection data are the means of five independent experiments, and mixed extracts results are the means of two independent experiments. The amount of total plasmid DNA was constant at 25 μg/75-cm^2 flask.

methioninemic members of family G contain, in only one of the two alleles, a G→A mutation at nt 791 that converts an arginine at position 264 to a histidine (R264H). Mutational analysis also revealed the presence of this single allelic R264 mutation in two additional hypermethioninemic individuals in a family, C. We further showed that the hypermethioninemic phenotype and the associated R264H mutation were also transmitted in a dominant fashion in family C. Both family G and C are of northern European origin but are not known to be related. Thus, dominant inheritance of hypermethioninemia correlates with this single allelic *MAT1A* mutation.

In earlier studies (Ubagai et al. 1995; Chamberlin et al. 1996), we showed that two individuals containing null *MAT1A* mutations have manifested neurological complications, including brain demyelination. However, hypermethioninemic individuals with *MAT1A* mutations that retain low levels of MAT activity have been free of clinical abnormalities. Therefore, it is not surprising that the hypermethioninemic individuals in both families G and C are clinically benign because this single allelic R264H mutation is predicted to retain ~30% of normal MAT I/III activity in the liver. Our study defines a threshold at which reduced MAT I/III activity leads to hypermethioninemia. Individuals with 50%–60% of MAT I/III activity appear to have normal or near normal plasma methionine levels (Mudd et al. 1995*b*; Chamberlin et al. 1996).

The dominant effect of the R264H mutation suggests that substitution of histidine for arginine at position 264 may reduce or abolish enzymatic activity of MATα1 oligomers formed between R264H mutant and R264 wild-type subunits. Two lines of evidence suggest that residue 264 is involved in salt-bridge formation essential for subunit dimerization and optimal MAT activity. First, expression of wild-type *MAT1A* cDNA in *E. coli* yielded enzymatically active MAT, and the encoded MATα1 subunit readily forms dimers. Substitution of histidine, a weakly basic amino acid, for arginine at position 264 markedly diminished the ability of the mutant R264H-MATα1 subunits to dimerize and resulted in almost a total loss of catalytic activity (<1% of wild-type activity). Moreover, mutant MATα1 subunits (R264L, R264D, and R264E) lacking a positive charge at residue 264 were incapable of forming dimers and were enzymatically inactive. In contrast, R264K-MATα1, a mutant maintaining a strong positive charge at position 264, forms dimers readily and retains ≥20% of wild-type MAT activity. Second, crystallographic studies of bacterial MAT (Takusagawa et al, 1996*a*, 1996*b*) have revealed that, in the central region of the MATα1 dimer, the subunits interact through polar interactions involving a salt bridge between R-244 of one subunit and E-42 and T-242 of the other. R-244, E-42, and T-242 in *E. coli* MAT correspond to R-264, E-57, and T-262 in human MATα1, and these three amino acids are conserved across species (Sakata et al, 1993). Our data strongly suggest that an intersubunit salt bridge between R-264 and E-57/T-262 in human MAT stabilize the MATα1 dimers optimal for MAT catalysis.

Cotransfection of R264 wild-type and R264H mutant cDNAs into COS-1 cells showed that the observed MAT activity does not reflect the amount of transfected wild-type cDNA. This suggests that the R264 wild-type MATα1 subunit can dimerize with the R264H mutant subunit, but the resultant heterodimer retains little enzymatic activity. It is possible that an intersubunit salt bridge between R-264 (wild-type) and E-57/T-262 (R264H mutant) stabilizes polar interactions between a weakly basic H-264 in the mutant subunit and E-57/T-262 in the wild-type subunit. We are currently investigating why hetero-oligomers of R264/R264H retain little or no MAT catalytic activity.

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