MTABC3, a Novel Mitochondrial ATP-binding Cassette Protein Involved in Iron Homeostasis*§

Received for publication, January 19, 2000, and in revised form, February 23, 2000

Noboru Mitsuhashi‡, Takashi Miki‡, Hiroshi Senbongi§, Norihide Yokoš, Hideki Yano‡, Masaru Miyazaki, Nobuyuki Nakajima, Toshihiko Iwanaga**, Yuji Yokoyama‡‡, Takehiko Shibata §§, and Susumu Seino‡‡‡

From the ‡Department of Molecular Medicine, Chiba University Graduate School of Medicine, Inohana, Chuo-ku, Chiba 260-8670, Japan, the §Cellular and Molecular Biology Laboratory, Riken, Wako-shi, Saitama 351-0198, Japan, the ¶Department of Medical Genetics, Novo Nordisk Pharma, Chiba University School of Medicine, Inohana, Chuo-ku, Chiba 260-8670, Japan, the **Department of Surgical Organ-Pathophysiology, Chiba University Graduate School of Medicine, Inohana, Chuo-ku, Chiba 260-8670, Japan, the ††Laboratory of Anatomy, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan, and the ‡‡‡Department of Pediatrics, Okayama University Medical School, Shikata-cho, Okayama 700-8558, Japan

Atm1p, a mitochondrial half-type ATP-binding cassette (ABC) protein in Saccharomyces cerevisiae, transports a precursor of the iron-sulfur (Fe/S) cluster from mitochondria to the cytosol. We have identified a novel half-type human ABC protein, designating it MTABC3 (mammalian mitochondrial ABC protein 3). MTABC3 mRNA is ubiquitously expressed in all of the rat and human tissues examined. MTABC3 protein is shown to be present in the mitochondria, as assessed by immunoblot analysis and confocal microscopic analysis of subcellular fractions of Chinese hamster ovary cells stably expressing MTABC3. Accumulation of iron in the mitochondria, mitochondrial DNA damage, and respiratory dysfunction in the yeast ATMI mutant strain (atm1-1 mutant cells) were almost fully reversed by expressing MTABC3 in these mutant cells. These results indicate that MTABC3 is a novel ortholog of the yeast and suggest an important role in mitochondrial function. Interestingly, the human MTABC3 gene has been mapped to chromosome 2q36, a region within the candidate locus for lethal neonatal metabolic syndrome, a disorder of the mitochondrial function associated with iron metabolism, indicating that MTABC3 is a candidate gene for this disorder.

** ATP-binding cassette (ABC)**† proteins constitute one of the largest superfamilies of membrane proteins in both prokaryotic and eukaryotic organisms, and their general structures are well conserved in evolution (1, 2). In eukaryotes, most of the members of the ABC protein family function as ATP-dependent active transporters in the plasma membranes and the membranes of intracellular organelle, including the endoplasmic reticulum, vacuoles, peroxisome, and mitochondria (3–7). Some ABC proteins, however, function as ion channels or regulators of ion channels (2, 8, 9). Recently, mutations of ABC proteins have been shown to be responsible for various genetic diseases in man (10).

Mitochondria provide cells with energy for many biological functions by oxidative phosphorylation. Reactive oxygen species are by-products of respiration. Their interaction with free iron in mitochondria through the Fenton reaction could lead to oxidative damage to lipids, proteins, and DNA in mitochondria (11, 12), suggesting that iron homeostasis is crucial in the maintenance of mitochondrial function.

Atm1p was the first member of the ABC protein family identified in mitochondria (7), and it plays an important role in normal cellular growth and iron homeostasis (11, 13). Further analysis of Atm1p has shown that it transports the precursor of the Fe/S cluster from mitochondria to the cytosol (14). Because mutation of ATM1 results in mitochondrial dysfunction (11), mutations of human mitochondrial ABC proteins could be associated with various diseases. Although the complete genomic sequences of Saccharomyces cerevisiae and Escherichia coli predict the existence of 29 and 79 members of the ABC protein family, respectively (15, 16), only a few mitochondrial ABC proteins have been identified to date.

In the course of our search for human ABC proteins in the mitochondria, two mitochondrial ABC proteins were reported by other laboratories: ABC7, an ortholog of Atm1p (17), and M-ABC1, the function of which is not known (18). A mutation of the human ABC7 gene has been shown responsible for X-linked sideroblastic anemia and ataxia (XLSA/A) (19). Here, we report a third human mitochondrial ABC protein, designated MTABC3. MTABC3 has 31.1% identity to Atm1p and is shown to be involved in iron homeostasis and to play an important role in mitochondrial functions such as maintenance of respiratory function and mitochondrial DNA. The human MTABC3 gene has 19 exons in the protein-coding region and has been mapped to chromosome 2q36. Because the locus for lethal neonatal

* This work was supported by a grant from Research on Human Genome and Gene Therapy from the Ministry of Health and Welfare, Japan, and by grants-in-aid from the Ministry of Education, Science, Sports and Culture, Japan; a grant from Novo Nordisk Pharma Ltd.; and a grant from Yamanouchi Foundation for Research on Metabolic Disorders. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†† The on-line version of this article (available at http://www.jbc.org) contains the exon/intron boundaries of the MTABC3 gene. The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF076775 (human MTABC3 cDNA) and AB039353-AB039371 (human MTABC3 gene).

‡‡ Supported by a grant from Japan Science and Technology (CREST).

§ To whom correspondence should be addressed: Dept. of Molecular Medicine, Chiba University Graduate School of Medicine, 1-8-1, Inohana, Chuo-ku, Chiba 260-8670, Japan. Tel.: 81-43-226-2187; Fax: 81-43-221-7803; E-mail: seino@molmed.m.chiba-u.ac.jp.

1 The abbreviations used are: ABC, ATP-binding cassette; NBF, nucleotide binding fold; CHO, Chinese hamster ovary cell; EST, expressed sequence tag; PCR, polymerase chain reaction; bp, base pair(s); kb, kilobase(s); Mtp, mitochondrial protein; XLSA/A, X-linked sideroblastic anemia and ataxia; RH, radiation hybrid.
metabolic syndrome, a disorder of mitochondrial function associated with iron metabolism (20, 21), has been mapped to the same region, MTABC3 is a strong candidate gene for this disorder.

**EXPERIMENTAL PROCEDURES**

cDNA Cloning of Human MTABC3—The human EST data base at the National Center for Biotechnology Information was screened with a partial nucleotide sequence of ATM1 as a probe. As a result, two human cDNAs encoding an ABC protein (ABC7) and an ABC transporter-like protein (EST 45597) were found. A partial cDNA fragment of EST 45597 was amplified by polymerase chain reaction (PCR) using a human liver cDNA as a template. The sense and antisense primers used were 5′-TTC AGT GTG CCT GGA CCA AGG AGC ACA ATT TTG-3′ and 5′-AAG TAT GTG CTT GCC TGC CCG GCC AGA TGG-3′ for G628A and K928R, respectively in combination with oligonucleotides (5′-GTG ACC AGT TAC GTT TTC CT-3′ and 5′-TCT TTG AGA GGG AAG TGG CC-3′). (24). Expression of the Atm1p, MTABC3, or MTABC3 double mutants was achieved by transforming YM13-1c cells with the multi-copy plasmid pYES2 under control of the GAL1 promoter (25). Isolation of yeast mitochondria and mitochondrial DNA have been described previously (26, 27). Measurement of free iron in the mitochondria of yeast cells was performed as described previously (28). The presence of multiple mtDNA copies was assessed by Southern analysis using whole mitochondrial DNA and Leu2 (β-isopropylmalate dehydrogenase cDNA) as probe, respectively (11). Mitochondrial respiratory function was assessed by spontaneous petite formation (11). Approximately 5 × 10⁶ cells cultured in SCGly were inoculated into 5 ml of YPD medium, and the cells were incubated at 30 °C with vigorous shaking for 48 h. Aliquots of the culture were withdrawn and spread on YPGly and YPD plates at the indicated times (Fig. 4C). The rate of maintenance of mitochondrial respiratory function was calculated by dividing the number of colonies on YPGly plates by the number of colonies on YPD plates (11).

Cloning of the Human MTABC3 Gene—To clone the human MTABC3 gene, a partial human liver cDNA fragment of MTABC3 (nt 1784-nt 2407) was used as a probe for screening a a FIX II human genomic library. A p3′ nick-translated probe, approximately 0.9 × 10⁶ plaques were screened under highly stringent conditions. Two positive clones were subcloned into plasmid vectors pGEM-3Z (Promega) and sequenced in both directions using ABI automatic sequencer (ABI PRISM™). The deduced full-length protein was designated MTABC3.

RNA Blot Analysis—Total RNAs were purified from various freshly isolated tissues of adult Wistar rats and cell lines by the guanidinium thiocyanate-phenol extraction method (22). For RNA transfer blot, 10 μg of total RNA from the various tissues or cells were denatured with formaldehyde, electrophoresed on a 1% agarose gel, and transferred to nylon membranes (Hybond N°, Amersham Pharmacia Biotech). RNA blot analysis was also carried out with the Human Multiple Tissue Northern blot system (CLONTECH), according to the manufacturer's protocol. The blots were probed with a 5′p nick-translated 580-base pair (bp) human MTABC3 fragment. Hybridizations were carried out under the standard conditions. The nylon membranes were washed in 0.1× SSC and 0.1% SDS at 50 °C for 1 h and exposed to x-ray films at -80 °C for 72 h.

Establishment of Chinese Hamster Ovary (CHO) Cells Stably Expressing MTABC3—FLAG-tag was attached at the 3′ end of human MTABC3 cDNA in the pcDNA 3.1(−) vector (Invitrogen). The resultant plasmid vector was transfected into CHO cells by electroporation at 950 microfarads and 0.232 kV/cm. The cells were cultured in dMEM medium (Life Technologies, Inc.) with 10% fetal bovine serum in the presence of 400 μg/ml of Geneticin for 2 weeks. Ninety-six Geneticin-resistant cells were isolated and screened by genomic PCR and RNA and protein blot analyses.

Subcellular Fractionation—Discontinuous sucrose gradient fractionation of CHO cells stably expressing FLAG-tagged MTABC3 was performed with slight modifications (23). Postnuclear supernatant (1 ml) was applied to the top of the sucrose gradients (11 ml) and centrifuged at 55,000 × g for 2 h at 4 °C. Each fraction (1 ml) was collected from the top fraction (fraction 1) to the bottom fraction (fraction 12). Each postnuclear fraction containing 2–20 μg of protein was precipitated with 15% trichloroacetic acid, separated on 10% SDS-polyacrylamide gel, and subjected to immunoblot analysis.

Immunocytochemistry by Confocal Laser Microscopy—Immunofluorescence staining of CHO cells expressing FLAG-tagged MTABC3 was performed on collagen-coated coverslips. The cells were incubated for 30 min at 37 °C with 25 μm MitoTracker Red CMXRs (Molecular Probes) in complete medium, fixed with 4% paraformaldehyde, and permeabilized with 0.1% Triton X-100. Immunoreactivity of MTABC3 was detected with anti-FLAG antibody (Kodak) and fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, Inc.). Coverslips were mounted on glass slides using PermaFlour (LifeShaw) and examined using a Zeiss fluorescence microscope.

Yeast Strains and Media—The following S. cerevisiae strains were used in this study: YM13-1c (MATa ade5 leu2 ura3 trp1 atam 11-Δ (p- chlb24)), CO3775 (MATa ade5 leu2 ura3 trp1 can1 atm11), and IL186–187 (MATa his1 trp1 can1 atm11 p- chlb24 aat2), as described previously (11). Yeast cells were grown in medium consisting of 1% yeast extract, 2% Bacto peptone (Difco), and 2% glucose (YPD) or 3% glycerol (SCGly). For selective growth, yeasts were cultured in a medium of 0.67% yeast nitrogen base without amino acids, 0.077% CSM URA (BIO101), and 3% glycerol (SCGly). Solid media were prepared by adding 2% Bacto agar (Difco) to the liquid media described above.

Analysis of MTABC3 Function—The Walker A-encoding region of MTABC3 cDNA was mutated by a PCR-based method using oligonucleotides bearing mismatched bases at the residues to be mutated (5′-CCA TCT GGG GCA GGG AGG AGC ACA ATT TTG-3′ and 5′-AAG TAT GTG CTT GCC TGC CCG GCC AGA TGG-3′) for G628A and K928R, respectively in combination with oligonucleotides (5′-GTG ACC AGT TAC GTT TTC CT-3′ and 5′-TCT TTG AGA GGG AAG TGG CC-3′). Expression of the Atm1p, MTABC3, or MTABC3 double mutants was achieved by transforming YM13-1c cells with the multi-copy plasmid pYES2 under control of the GAL1 promoter (25). Isolation of yeast mitochondria and mitochondrial DNA have been described previously (26, 27). Measurement of free iron in the mitochondria of yeast cells was performed as described previously (28). The presence of multiple mtDNA copies was assessed by Southern analysis using whole mitochondrial DNA and Leu2 (β-isopropylmalate dehydrogenase cDNA) as probe, respectively (11). Mitochondrial respiratory function was assessed by spontaneous petite formation (11). Approximately 5 × 10⁶ cells cultured in SCGly were inoculated into 5 ml of YPD medium, and the cells were incubated at 30 °C with vigorous shaking for 48 h. Aliquots of the culture were withdrawn and spread on YPGly and YPD plates at the indicated times (Fig. 4C). The rate of maintenance of mitochondrial respiratory function was calculated by dividing the number of colonies on YPGly plates by the number of colonies on YPD plates (11).

Cloning of the Human MTABC3 Gene—To clone the human MTABC3 gene, a partial human liver cDNA fragment of MTABC3 (nt 1784-nt 2407) was used as a probe for screening a a FIX II human genomic library. A p3′ nick-translated probe, approximately 0.9 × 10⁶ plaques were screened under highly stringent conditions. Two positive clones were subcloned into plasmid vectors pGEM-3Z (Promega) and sequenced using an ABI automatic sequencer (ABI PRISM™). Exon-intron boundaries were determined by a comparison of genomic and cDNA sequences. Intron sizes were determined by sequencing or PCR amplification.
spectively, indicating that MTABC3 represents a new member of the half-type ABC protein subfamily.

Northern blot analysis revealed that human MTABC3 cDNA hybridized to the major transcript of ~3.4 kb expressed widely in rat tissues and various cell lines and at high levels in the testis, kidney, and cerebellum (Fig. 2A). In addition to the ~3.4-kb transcript, a transcript of ~4.0 kb was also detected in many tissues and at high levels in cerebellum. On the other hand, only a single transcript (~3.4 kb) of MTABC3 was detected in all of the human tissues examined, and it is expressed at high levels in the heart and skeletal muscles (Fig. 2B).

Because half-type ABC proteins are generally known to be present in intracellular organelle, we examined the subcellular localization of MTABC3. For this purpose, we established CHO cells stably expressing FLAG-tagged human MTABC3. The single immunoreactive protein MTABC3 was detected as an 80-kDa protein in fractions 9–12, peaking at fraction 10, which coincides with the major fraction of cytochrome c oxidase subunit IV (COX IV), a molecular marker for mitochondrial fractions (Fig. 2C). In contrast, Na/K-ATPase subunit α, a molecular marker of microsomal fractions, is present in fractions 5–11, peaking at fractions 6 and 7. To confirm that MTABC3 is present in the mitochondria, CHO cells expressing MTABC3 were stained with anti-FLAG antibody and MitoTracker Red CMXRos, a stain specific for mitochondria (35), and observed under confocal laser microscopy. Immunostaining of MTABC3 revealed that it is precisely co-localized with granular worm-like mitochondrial structures (Fig. 2D).

The role of MTABC3 in the mitochondrial function was then investigated. In previous studies, functional analyses of Atm1p and ABC7 were performed using an ATM1 disruptant that is completely defective in ATM1 function (13, 17). In the present study, we used atm1-1 mutant cells that are partially defective in Atm1p function (11). The atm1-1 mutant cells showed a 50-fold higher level of free iron accumulation relative to that found in the mitochondria of wild-type yeast cells, mitochondrial genome instability, and loss of mitochondrial respiratory function (11). We then investigated whether these phenotypic alterations of atm1-1 mutant cells could be reversed by introducing MTABC3 into atm1-1 mutant cells. The mitochondria were purified, and their iron content was determined (Fig. 3A). The amount of free iron in mitochondria of atm1-1 mutant cells in which the control plasmid (pYES2) alone was introduced was 30.02 ± 3.96 nmol/mg mitochondrial protein (Mtp). On the other hand, the amount of free iron in the mitochondria of atm1-1 mutant cells transformed with Atm1p or MTABC3 was 11.88 ± 0.91 or 11.34 ± 0.72 nmol/mg Mtp, respectively, with both values approximately one-third of the value found in atm1-1 mutant cells. To determine whether the compensation is specific to MTABC3 function, we examined the effect of the MTABC3 mutant on free iron content in mitochondria. Mutations of the glycine (G) and lysine (K) residues within the Walker A motif, respectively, indicated that MTABC3 represents a new member of the half-type ABC protein subfamily.

Northern blot analysis revealed that human MTABC3 cDNA hybridized to the major transcript of ~3.4 kb expressed widely in rat tissues and various cell lines and at high levels in the testis, kidney, and cerebellum (Fig. 2A). In addition to the ~3.4-kb transcript, a transcript of ~4.0 kb was also detected in many tissues and at high levels in cerebellum. On the other hand, only a single transcript (~3.4 kb) of MTABC3 was detected in all of the human tissues examined, and it is expressed at high levels in the heart and skeletal muscles (Fig. 2B).

Because half-type ABC proteins are generally known to be present in intracellular organelle, we examined the subcellular localization of MTABC3. For this purpose, we established CHO cells stably expressing FLAG-tagged human MTABC3. The single immunoreactive protein MTABC3 was detected as an 80-kDa protein in fractions 9–12, peaking at fraction 10, which coincides with the major fraction of cytochrome c oxidase subunit IV (COX IV), a molecular marker for mitochondrial fractions (Fig. 2C). In contrast, Na/K-ATPase subunit α, a molecular marker of microsomal fractions, is present in fractions 5–11, peaking at fractions 6 and 7. To confirm that MTABC3 is present in the mitochondria, CHO cells expressing MTABC3 were stained with anti-FLAG antibody and MitoTracker Red CMXRos, a stain specific for mitochondria (35), and observed under confocal laser microscopy. Immunostaining of MTABC3 revealed that it is precisely co-localized with granular worm-like mitochondrial structures (Fig. 2D).

The role of MTABC3 in the mitochondrial function was then investigated. In previous studies, functional analyses of Atm1p and ABC7 were performed using an ATM1 disruptant that is completely defective in ATM1 function (13, 17). In the present study, we used atm1-1 mutant cells that are partially defective in Atm1p function (11). The atm1-1 mutant cells showed a 50-fold higher level of free iron accumulation relative to that found in the mitochondria of wild-type yeast cells, mitochondrial genome instability, and loss of mitochondrial respiratory function (11). We then investigated whether these phenotypic alterations of atm1-1 mutant cells could be reversed by introducing MTABC3 into atm1-1 mutant cells. The mitochondria were purified, and their iron content was determined (Fig. 3A). The amount of free iron in mitochondria of atm1-1 mutant cells in which the control plasmid (pYES2) alone was introduced was 30.02 ± 3.96 nmol/mg mitochondrial protein (Mtp). On the other hand, the amount of free iron in the mitochondria of atm1-1 mutant cells transformed with Atm1p or MTABC3 was 11.88 ± 0.91 or 11.34 ± 0.72 nmol/mg Mtp, respectively, with both values approximately one-third of the value found in atm1-1 mutant cells. To determine whether the compensation is specific to MTABC3 function, we examined the effect of the MTABC3 mutant on free iron content in mitochondria. Mutations of the glycine (G) and lysine (K) residues within the Walker A motif,
A Novel Mitochondrial ABC Protein

FIG. 3. Functional analysis of MTABC3. A, iron content of mitochondria isolated from yeast YM13-1c strain transformed with pYES2, ATM1, MTABC3, or MTABC3 double mutant (G628A, K629R) (values are mean ± S.E.). B, Southern blot analysis of mitochondrial DNA. The mitochondrial DNAs (MtDNA) of wild-type yeast (JL166–187) and pYES2 yeast (CG378 pYES2) were used as controls; mitochondrial DNA in each transformed yeast type is shown. C, the rates (% of colonies maintaining mitochondrial respiratory function obtained by transforming pYES2 vector (■), ATM1 (■), MTABC3 (○), or MTABC3 double mutant G628A, K629R (●) into atm1-1 mutant cells at the indicated times are shown (mean ± S.E.). The rates were calculated by dividing the number of colonies on YPGly plates by the number of colonies on YPD plates. The values are the average of three independent experiments.

FIG. 4. Genomic organization and chromosomal localization of human MTABC3. A, human MTABC3 gene spans ~11 kb and contains 19 exons in the protein-coding region. Exons are indicated by filled boxes. The translation initiation and termination codons are indicated by ATG and TGA, respectively. Scale bars for exon and intron sizes are indicated. B, fluorescence in situ hybridization of human MTABC3. The arrow indicates the signals on chromosome 2q36. C, radiation hybrid mapping of human MTABC3. Distances between STSs are given in cR 10000 on a Stanford G3 panel. MTABC3 was mapped to the region between D2S1297 and SHGC-32531. The genetic map is derived from Visapa et al. (21).

- Oxidative stress impairs various mitochondrial functions, including the respiratory function (40, 41). The respiratory function has been shown to be severely impaired in atm1-1 mutant cells as assessed by petite formation (11). We tested whether respiratory function could be maintained in atm1-1 mutant cells transformed with the pYES2 vector alone, whereas the amount of mitochondrial DNA in atm1-1 mutant cells transformed with Atm1p or MTABC3 is similar to the level in wild-type yeast cells.

- It has been shown that mitochondrial iron accumulation causes damage to mitochondrial DNA (11, 12). To assess this damage, Southern blot analysis of mitochondrial DNA was carried out (Fig. 3B). There is little mitochondrial DNA in atm1-1 mutant cells transformed with the pYES2 vector alone, whereas the amount of mitochondrial DNA in atm1-1 mutant cells transformed with Atm1p or MTABC3 is similar to the level in wild-type yeast cells.

- Oxidative stress impairs various mitochondrial functions, including the respiratory function (40, 41). The respiratory function has been shown to be severely impaired in atm1-1 mutant cells as assessed by petite formation (11). We tested whether respiratory function could be maintained in atm1-1 mutant cells transformed with the pYES2 vector alone, whereas the amount of mitochondrial DNA in atm1-1 mutant cells transformed with Atm1p or MTABC3 is similar to the level in wild-type yeast cells.

- We have identified a novel human ABC protein present in the mitochondria, designated MTABC3. Atm1p, which was identified in S. cerevisiae, was the first member of the ABC protein family to be found in mitochondria (7). Atm1p is located in the mitochondrial inner membrane and functions as an exporter of Fe/S clusters (14), suggesting that Atm1p could play a critical role in iron homeostasis in mitochondria. ABC7 and M-ABC1, two other human ABC proteins present in the mitochondria, have recently been reported (17, 18). ABC7 has been shown to be involved in iron homeostasis, but the functional role of M-ABC1 is not known. Because there is 31% amino acid identity between human MTABC3 and yeast Atm1p, human MTABC3 could be a functional ortholog of yeast Atm1p. In atm1-1 mutant cells (11), there is accumulation of free iron in the mitochondria, mitochondrial DNA damage, and respiratory dysfunction. To investigate the functional roles of MTABC3, we transformed atm1-1 mutant cells with human MTABC3. We found that all the phenotypic consequences in atm1-1 mutant cells can be reversed by the expression of human MTABC3 in the cells, supporting the possibility that MTABC3 is a functional ortholog of Atm1p. Interestingly, although the iron accumulation is only partially reversed (Fig. 3A), the damage of mitochondrial DNA (Fig. 3B) is almost fully reversed by the double mutant MTABC3 (G628A, K629R) in atm1-1 mutant cells. This indicates that the degree of mitochondrial DNA damage does not parallel the accumulation of iron in the mitochondria, suggesting that the mitochondrial DNA damage is a defect secondary to the oxidative damage caused by the accumulation of iron in the mitochondria (11, 12). ABC7 and MTABC3 can both substitute for Atm1p in yeast. However, XLSA/A is caused by a mutation of the ABC7 gene alone (19), indicating that MTABC3 cannot substitute for ABC7 and that the function of MTABC3 is different from that of ABC7. Although all characterized half-type ABC proteins function as dimers, it is not known at present whether MTABC3 functions as a homodimer or a heterodimer with ABC7 or unidentified half-type ABC proteins.

- MTABC3 has only one NBF, indicating that, like Atm1p and ABC7, it belongs to the half-type ABC protein subfamily. It has been suggested that both Atm1p and ABC7 have six putative membrane-spanning regions, with the NBF of both proteins located in the matrix of the mitochondria (7, 16). Considering the functional similarity between ABC7 and MTABC3, the NBF of MTABC3 may also be located in the matrix of the mitochondria. Although hydrophathy analysis using the PSORT program (28) suggests the presence of eight putative membrane-spanning regions in MTABC3, the details of membrane topology must await further biochemical analysis. All of the
ABC proteins identified to date in the intracellular organelle of mammalian cells are half-type ABC proteins (1, 10, 17, 18). Mutations of ABC proteins are known to cause many disorders, including cystic fibrosis, persistent hyperinsulinemic hypoglycemia of infancy, and adrenoleukodystrophy (3, 42, 43).

Like a mutation of the \( \text{MTABC3} \) gene in XLSA/A (19), mutations of \( \text{MTABC3} \) might also be associated with disorders of iron metabolism. As a first step in determining the role of the \( \text{MTABC3} \) gene in the development of genetic disorders, we cloned the \( \text{MTABC3} \) gene and determined the exon/intron boundaries (available as supplementary information in the on-line version of this article). The human \( \text{MTABC3} \) gene spans ~11 kb and has 19 exons in the protein-coding region (Fig. 4A). Fluorescence in situ hybridization reveals that the human \( \text{MTABC3} \) gene is located at chromosome 2q36 (Fig. 4B). Radiation hybrid mapping further narrows the region to between D2S1297 and SHGC-32531 (Fig. 4C). Interestingly, lethal neonatal metabolic syndrome, a disorder of mitochondrial function associated with iron metabolism, has been mapped between D2S164 and D2S163 (20, 21). Thus, the human \( \text{MTABC3} \) gene is a strong candidate for the causal gene in this disorder. Cloning of the human \( \text{MTABC3} \) CDNA and gene and its functional characterization should facilitate studies of its roles in mitochondrial function as well as in the development of lethal neonatal metabolic syndrome.

**Acknowledgments**—We thank Dr. M. Maekawa for helpful advice during the course of this study. We also thank A. Tamamoto, A. Igawa, and A. Saraya for excellent technical assistance.

**REFERENCES**

1. Higgins, C. F. (1992) *Annu. Rev. Cell Biol.* 8, 67–113
2. Higgins, C. F. (1995) *Cell* 82, 693–669
3. Riordan, J. R., Rommens, J. M., Kerem, Bat-Sheva., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenksi, J., Lok, S., Plavsic, N., Chou, J.-L., Drumm, M. L., Iannuzzi, M. C., Collins, P. S., and Tsui, L.-C. (1989) *Science* 245, 1066–1073
4. Monaco, J. J. (1992) *Annu. Rev. Cell Biol.* 1, 593–638
5. Oritz, D. F., Kreppel, L., Speiser, D. M., Scheel, G., McDonald, G., and Ow, D. W. (1992) *EMBO J.* 11, 3491–3499
6. Kamijo, K., Taketani, S., Yokota, S., Osumi, T., and Hashimoto, T. (1990) *J. Biol. Chem.* 265, 4534–4540
7. Leighton, J., and Schatz, G. (1995) *EMBO J.* 14, 188–195
8. Welsh, M. J., Anderson, M. P., Rich, D. P., Berger, H. A., Denning, G. M., Ostedgaard, L. S., Sheppard, D. N., Cheng, S. H., Gregory, R. J., and Smith, A. E. (1992) *Neuron* 8, 821–829
9. Inagaki, N., Gono, T., Clement, J. P. 4th., Namba, N., Inazawa, J., Gonzalez, G., Aguilar-Bryan, L., Seino, S., and Bryan, J. (1995) *Science* 270, 1166–1170
10. Dean, M., and Allikmets, R. (1995) *Curr. Opin. Genet. Dev.* 5, 779–785
11. Senhong, H., Ling, F., and Shibata, T. (1999) *Mol. Gen. Genet.* 262, 426–436
12. Stoba, S. J., and Bagchi, D. (1999) *Free Radical Biol. Med.* 18, 321–336
13. Kispal, G., Csere, P., Guiard, B., and Lill, R. (1997) *FEBS Lett.* 418, 346–350
14. Kispal, G., Csere, P., Prohl, C., and Lill, R. (1999) *EMBO J.* 18, 3981–3989
15. Linton, K. J., and Higgins, C. F. (1988) *Mol. Microbiol.* 2, 5–13
16. Decottignies, A., and Goffeau, A. (1997) *Nat. Genet.* 15, 137–145
17. Csere, P., Lill, R., and Kispal, G. (1998) *FEBS Lett.* 427, 266–270
18. Hugue, D. L., Liu, L., and Ling, V. (1999) *J. Biol. Chem.* 285, 379–389
19. Allikmets, R., Baskind, W. H., Hutchinson, A., Schureck, N. D., Dean, M., and Koehler, D. M. (1999) *Hum. Mol. Genet.* in press, 743–749
20. Fellman, V., Rapola, J., Pihko, H., Varilo, T., and Raivio, K. O. (1998) *Lancet* 351, 490–493
21. Visappia, I., Fellman, V., Varilo, T., Palotie, A., Raivio, K. O., and Peltonen, L. (1998) *Ann. Hum. Genet.* 63, 1396–1403
22. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* 162, 156–159
23. Wendland, B., and Scheller, B. H. (1994) *Mol. Endocrinol.* 8, 1070–1082
24. Higuchi, R., Krummel, B., and Saiki, R. K. (1988) *Nucleic Acids Res.* 16, 7351–7367
25. Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983) *J. Bacteriol.* 153, 163–168
26. Daum, G., Bohni, P. C., and Schatz, G. (1982) *J. Biol. Chem.* 257, 13028–13033
27. Houdusheh, M. E. S., Shumard, D. S., Tatti, K. M., and Grossman, L. I. (1980) *Biochim. Biophys. Acta* 610, 221–228
28. Tangeras, A., Flatmark, T., Backstrom, D., and Ehrenberg, A. (1980) *Biochim. Biophys. Acta* 599, 162–175
29. Takahashi, E., Hori, T., O’Connell, P., Leppert, M., and White, R. (1990) *Hum. Genet.* 86, 14–16
30. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) *J. Mol. Biol.* 215, 403–410
31. Savary, S., Allikmets, R., Denizot, F., Luciani, Marie-Françoise., Mattei, Marie-Genevière., Dean, M., and Chiniomi, G. (1997) *Genomics* 41, 275–278
32. Nakai, K., and Kanehisa, M. (1992) *Genomics* 14, 897–911
33. Bateman, A., Birney, E., Durbin, R., Eddy, S. R., Finn, R. D., and Sonnhammer, E. L. (1999) *Nucleic Acids Res.* 27, 260–262
34. Shimada, Y., Okuno, S., Kawai, A., Shimoyama, H., Saito, A., Suzuki, M., Ono, Y., Nishino, N., Kanemoto, N., Fujiwara, T., Horie, M., and Takahashi, E. (1998) *J. Hum. Genet.* 43, 115–122
35. Chen, Q., Lin, Reih-Yi., and Rubin, C. S. (1997) *J. Biol. Chem.* 272, 15247–15257
36. Anderson, P. A., and Walsh, M. J. (1992) *Science* 257, 1701–1704
37. Azzaria, M., Schurr, E., and Gros, P. (1989) *Mol. Cell. Biol.* 9, 5289–5297
38. Gribble, F. M., Tucker, S. J., and Ashcroft, F. M. *EMBO J.* 16, 1145–1152
39. Berkower, C., and Michaelis, S. (1991) *EMBO J.* 10, 3777–3785
40. Del Maestro, R. F. (1980) *Acta Physiol. Scand.* Suppl. 492, 153–168
41. Ames, B. N., Shigenaga, M. K., and Hagen, T. M. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 7915–7922
42. Thomas, P. M., Cote, G. J., Wahlk, N., Haddad, B., Mathew, P. M., Rahl, W., Aguilar-Bryan, L., Gabriel, B. F., and Bryan, J. (1995) *Science* 268, 426–429
43. Mosser, J., Douar, Anne-Marie., Sarde, Claude-Olivier., Kioschis, P., Feil, R., Moser, H., Pouatka, Anne-Marie., Mandel, Jean-Louis., and Aubourg, P. (1993) *Nature* 361, 726–730