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Expression of Tom34 Splicing Isoforms in Mouse Testis and Knockout of *Tom34* in Mice

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The 34-kDa translocase of the outer mitochondrial membrane (Tom34) is a putative mammalian-specific factor involved in protein import into mitochondria. We analyzed the genomic sequence of the mouse *Tom34* gene and found it has two alternative initial exons. Using reverse transcription and the polymerase chain reaction (RT-PCR), we found that these two mRNAs differs only in the 5′-proximal sequences corresponding to the two initial exons (exon 1a and 1b). Tom34 mRNA with exon 1a (Tom34a) is expressed ubiquitously, while that with exon 1b (Tom34b) is expressed only in mature testicular germ cells. To explore the *in vivo* function of Tom34 proteins, we generated *Tom34*-deficient mice by targeted disruption. The *Tom34*$^{-/-}$ mice were viable and grew normally and had a normal Mendelian inheritance pattern. Male as well as female *Tom34*$^{-/-}$ mice were fertile. *In vitro*-preprotein import into isolated mitochondria showed no apparent difference between *Tom34*$^{-/-}$ and wild-type mice. These results indicate that Tom34 is dispensable for mouse growth and development under optimal conditions.

Key words: mice, mitochondria, preprotein, testis, translocase.

Abbreviations: BLAST, Basic Local Alignment Search Tool; RT-PCR, reverse transcription and polymerase chain reaction; Tom, the translocase of the outer mitochondrial membrane.

The fundamental mitochondrial translocation machinery for preprotein import has been isolated and well characterized in lower eukaryotes, *Saccharomyces cerevisiae* and *Neurospora crassa* (*1*, *2*). At an early step of translocation, preproteins synthesized in the cytosol are recognized by a dynamic protein complex, termed the translocase of the outer membrane of mitochondria (Tom). Among various Tom components, the receptor components for the preproteins form heterodimeric subcomplexes (Tom70-Tom37 and Tom20-Tom22). Tom70 contains seven tetratricopeptide repeats (TPR), and Tom20 and Tom37 contain a single copy of this motif. The two receptor subcomplexes interact via TPRs of Tom20 and Tom70 (*3*).

The presence of the homologs of the mammalian Tom receptor components, Tom70 (*4*, *5*), Tom20 (*6–8*), and Tom22 (*9*, *10*), suggests that these are of fundamental importance in the import mechanisms (*11*). However, several novel receptor candidates are found in mammalian mitochondria. Mouse metaxin is a mitochondrial outer membrane protein that shows significant sequence identity to yeast Tom37, and antibodies against metaxin partially inhibited the translocation of preadrenodoxin into mitochondria (*12*). Antibodies against OM37, a 37-kDa outer membrane protein of mitochondria, also inhibited mitochondrial docking and subsequent transport of preadrenodoxin into mitochondria (*13*). Human Tom34 was identified by screening for a degenerate TPR motif found in many Tom proteins (*14*). It has no counterpart among other Tom proteins in lower eukaryotes. Tom34 associates with the mitochondrial outer membrane, but mostly resides in cytosol (*15*). Two different preparations of antibodies against Tom34 inhibited preprotein import into isolated mitochondria (*14*, *15*). Furthermore, *in vitro* import of preproteins was strongly inhibited by an NH_2-terminal-deleted fragment of human Tom34 protein (*15*). In accord with its role in mitochondrial preprotein import, Tom34 was shown to interact with the mature portion of some preproteins and proposed to be a molecular chaperone that keeps preproteins in an unfolded, import-competent state (*16*). Using a yeast two-hybrid screen, two different groups identified Tom34 binding partners as hsp90 (*17*), ATPase-related valosin-containing protein and the lysosomal H^+-transporting ATPase member M (*18*).

Besides a role in general mitochondrial protein import, rat and human Tom34s are highly expressed in some tissues including testis and brain (*15*), suggesting that Tom34 may have a special function in these tissues.

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To clarify the function of the Tom34, especially in testis, we analyzed its expression in testis, determined its genomic sequence and generated *Tom34*-deficient mice.

MATERIALS AND METHODS

Isolation of Tom34 Gene—To construct a *Tom34*-deficient mouse, a C57BL/6 mouse liver EMBL3 SP6/T7 phage genomic library (1×10^6 plaque units) was screened with a mouse EST clone (nucleotide 42–283; GenBank Accession Number AA065497) that was homologous to a region around the translation start site of human Tom34 cDNA. Two phage clones out of nine positive clones were selected and sequenced using a Dye terminator sequencing kit and a 373A autosequencer (Applied Biosystems). The distal half of the Tom34 gene was amplified with TaKaRa LA Taq DNA polymerase (Takara Shuzo, Kyoto) from mouse liver genomic DNA. The nucleotide sequence segment that lies in the 3′-untranslated region was amplified by an inverse PCR technique (*19*, *20*). The amplified fragments were cloned into a TA cloning vector (Promega), and their sequences were determined.

Generation of Tom34-Deficient Mice—In constructing the targeting vector, the *neo* cassette was placed between the *Msc* I site of exon 1a and the *Bss* HII site of exon 1b of the *Tom34* gene. Lengths of the homologous regions were 4.6 kb and 3.8 kb in the targeting vector at the 5′ and 3′ sides of the *neo* cassette, respectively. The targeting vector was linearized with *Not*I and introduced into TT2 ES cells derived from an F_1 embryo between C57BL/6 and CBA mice (Charles River) by electroporation as previously described (*21*, *22*). Homologous recombinant ES clones were initially identified by PCR and their genotype was confirmed by Southern blot analysis. Polymerase chain reaction (PCR) primers used were 5′-CGCTGCACGGTTGGAGTACAGAACA-3′ as the 3′-side primer in the Tom34 gene and 5′-ATCGCCTTCTTGACGAGTTCTTCTG-3′ as the 5′-side primer in the *neo* gene. Genomic DNAs extracted from ES cells were digested with *Sac*I, and Southern blot analysis was performed. A 423-bp fragment of the *Tom34* gene (nucleotide 8973–9395, GenBank Accession Number AB087254) that was proximal to the 3′-end of the homologous region of the targeting vector and a *Pst*I–*Bam*HI-digested 614-bp fragment of the *neo* gene were used as probes for the analysis. Two mutant mouse strains were generated from two independent homologous recombinant ES clones of the disruption. The genotype of each mutant mouse was routinely determined on a tail tissue sample by PCR. To detect normal and mutant alleles, primers 5′-GTTGACCACGAGTTCCAGACAGA-3′ and 5′-TGGGATTCCTCATTTACAGGTTG-3′ were used. To detect the mutant allele, primers 5′-ATCGCCTTCTTGACGAGTTCTTCTG-3′ and 5′-TGGGATTCCTCATTTACAGGTTG-3′ were used. The genotype was confirmed by Southern blot analysis. The mice were housed in an environmentally controlled room of the Center for Animal Resources and Development in Kumamoto University under the guidelines of Kumamoto University for animal and recombinant DNA experiments.

Reverse Transcription and Polymerase Chain Reaction (RT-PCR) Analysis—RT-PCR analysis was performed with total RNAs using a One-step NA PCR kit (Takara Shuzo) according to the manufacturer's protocol. The sequences of the primers 1a, 1b, and 2 were: 5′-TAAAGGCACGCAGCCCTAGC-3′, 5′-TCTACCATCACCACCCCCCA-3′, and 5′-AAACAGGCACCACAGGGATAG-3′, respectively.

Northern Blot Analysis—Total RNA was electrophoresed in formaldehyde-agarose gels, and blotted onto nylon membranes. A digoxigenin-labeled antisense RNA probe was synthesized from mouse Tom34 cDNA (nucleotide 196–1190; GenBank Accession Number BC018278) under the control of the T7 promoter using a transcription kit (Roche Diagnostics, Tokyo). Chemiluminescence signals were captured with a LAS1000plus image analyzer (Fuji Photo Film, Tokyo).

Histological and Immunohistochemical Analysis—Male mice (10 weeks old) were deeply anesthetized and perfused with ice-cold PBS, and a fixative solution consisting of 4% paraformaldehyde and PBS was given through the ascending aorta. The testes were immersed in the fixative solution for 4 h at 4°C, washed in PBS for 4 h, dehydrated through a graded series of ethanol and xylene, and embedded in a paraffin block. Sections of 5 μm in thickness were deparaffinized and stained with a haematoxylin-eosin solution. For immunohistochemical analysis, deparaffinized sections were digested with 0.05% trypsin at 37°C for 10 min to increase the antigenicity. The digested sections were pretreated with 5 mM periodic acid for 30 min to inhibit endogenous peroxidase activity. The sections were incubated for 2 h with antiserum against human Tom34 (*15*) at 1:100 dilution. An avidin-biotin kit (Vector Laboratories, Burlingame, CA) was used for first antibody detection. Peroxidase activity was visualized using 3, 3′-diaminobenzidine as a substrate.

Other Methods—The testicular cells were fractionated by use of an elutriator as described (*23–26*). Preparation of mouse (3 months old) testis mitochondria and *in vitro* import analysis were carried out as described previously (*27*).

RESULTS

Genomic Organization of the Mouse Tom34 Gene—To generate a *Tom34*-deficient mouse, we determined the Tom34 genomic sequence (GenBank Accession Number AB087254). As shown in Fig. 1A, the mouse *Tom34* gene is 24.7 kb long with seven exons. However, there are two possible initial exons with very similar coding sequences. We designated these exons as exon 1a and exon 1b (Fig. 1B). The coding sequences of exon 1a and exon 1b are 83% identical. The predicted amino acid sequences of these exons are 91% identical (38 of 42 residues matched). Furthermore, BLAST (Basic Local Alignment Search Tool) search of mouse expressed-sequence tag (EST) database with the Tom34 genomic sequence revealed that both exons are alternatively transcribed and joined to the same exon 2 (Fig. 1C). Thus, the *Tom34* gene is transcribed to generate two cDNAs of Tom34. Many more EST clones contained the sequence corresponding to exon 1a than that of exon 1b. Thus, exon 1b may be differentially transcribed in specific tissue(s) or cell type(s).

Expression of Tom34 in Various Tissues—Expression of Tom34 mRNAs in various tissues was analyzed by

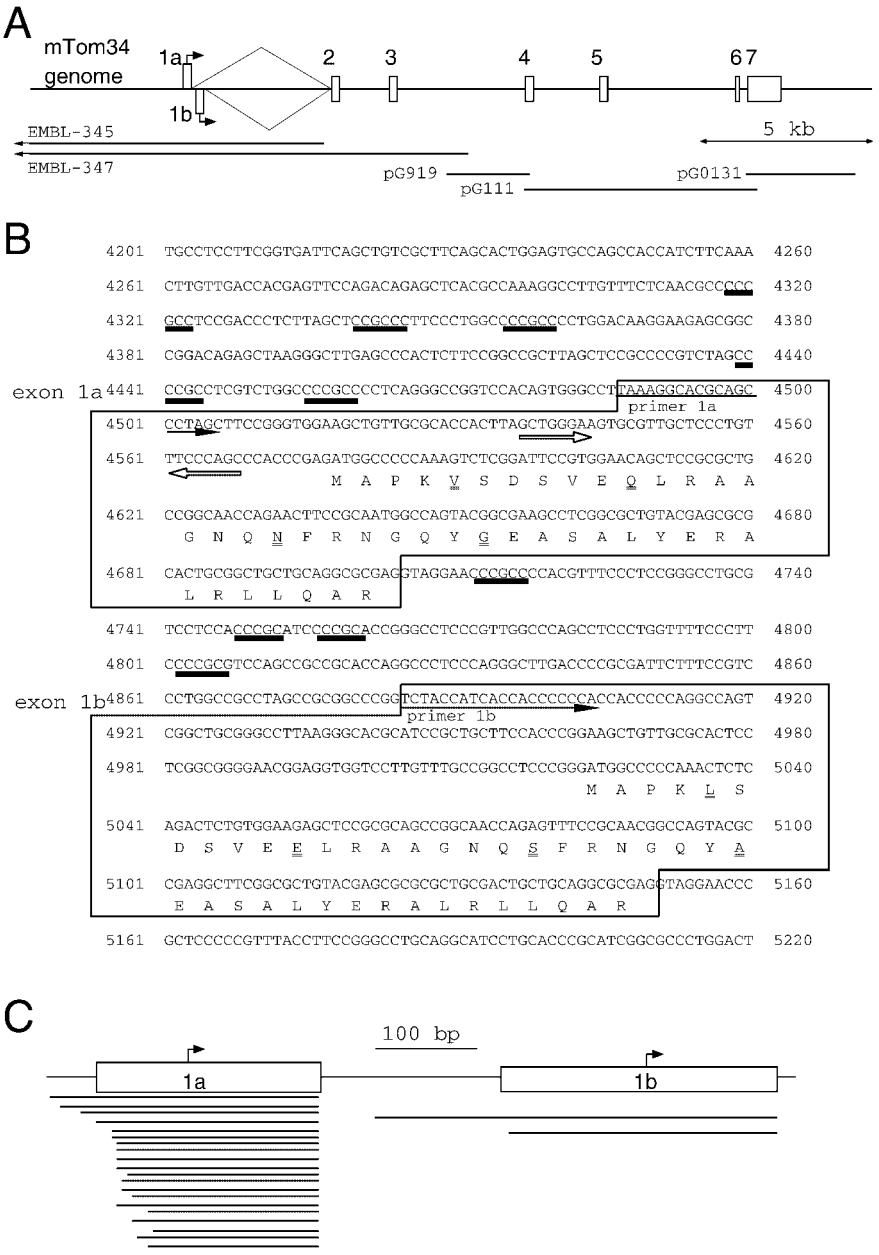

Fig. 1. **Structure of the mouse Tom34 Gene.** (A) Schematic diagram of the *Tom34* gene from C57BL/6 mouse. Exons are indicated by rectangles. The two exons encoding initiation methionines are designated as exon 1a and exon 1b. Arrows indicate translation start sites. Relative positions of two phage clones (EMBL-345 and -347) and three plasmid clones (pG919, pG111, and pG0131) are indicated by lines. (B) Nucleotide sequence around exon 1a and exon 1b. Putative exon regions are boxed. Underlined sequences represent GC boxes. Double underlined-residues denote positions of different residues between exon 1a and 1b. Solid arrows represent forward primers used for RT-PCR analysis. Open arrows represents an 8-bp stem of a hairpin structure located in the 5′-untranslated region of exon 1a. Numbers of nucleotides are those of the genomic sequence in the database (GenBank Accession Number AB087254). (C) Results of BLAST (Basic Local Alignment Search Tool) search analysis on mouse expressed-sequence tagged (EST) database. Each line represents the position of a corresponding clone. The 3′-flanking regions of all the EST clones are joined directly to the sequence corresponding to exon 2.

Northern blot analysis (Fig. 2A). When a 1-kb cDNA covering exons 2 to 7 was used as a probe, a single intense band of 2.2 kb was observed in testes. Tom34 mRNA was not separated into multiple species by longer gel electrophoresis (data not shown). Other tissues showed lower expression. Tissue distribution of Tom34 protein correlated well with that of mRNA. Differences in Tom34 expression in mouse tissues were more prominent than in human and rat tissues (*15*).

We next examined expression of exon 1a and exon 1b in several tissues. As Tom34 mRNA and proteins containing exon 1a and exon 1b could not be separated by Northern blot analysis and Western blot analysis, we used reverse transcription and polymerase chain reaction (RT-PCR) to detect expression of Tom34 mRNAs with exon 1a and exon 1b (Fig. 2B). Two forward primers (primer 1a and 1b in Fig. 1B) were designed to detect expression of Tom34 cDNAs with exon 1a and exon 1b. As a reverse primer, primer 2 located in exon 4 was used for amplification. Only Tom34 mRNA containing exon 1a was detected in brain, kidney and liver, whereas both mRNA species containing exon 1a or exon 1b were present in testis. Sequence analysis of these two species revealed that they are Tom34 cDNAs transcribed from exon 1a or from exon 1b. The 3′ regions of both cDNAs were identical to the predicted sequence of exon 2 to 4. This result indicates that two mRNAs for Tom 34 are present in testis. One contains exon 1a (designated as Tom34a) and the other contains exon 1b (Tom34b). An EST clone with exon 1b was derived from a testis library (Fig. 1C), and our RT-PCR results indicate highly selective expression of the Tom34b cDNA in testis.

Two Alternative Forms of Tom34 cDNA in Testis—Expression of the two alternative forms of Tom34 mRNA was analyzed in testes from pubertal stages of wild-type BALB/c mice by RT-PCR (Fig. 3A). The Tom34b mRNA

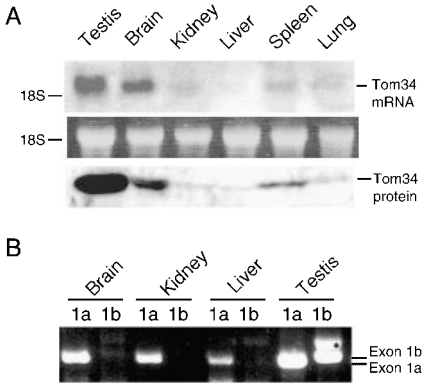

Fig. 2. **Expression of Tom34 mRNA and protein (A) and exon 1a and exon 1b (B) in various tissues.** (A) Northern blot analysis of Tom34 mRNA (upper panel), ethidium bromide staining of 18S rRNA (middle panel) and Western blot analysis of Tom34 protein (lower panel). Total RNA samples (2 μg each) from various tissues from 8-week BALB/c mouse were subjected to Northern blot analysis, using digoxigenin-labeled RNA as a probe. Integrity of the RNA samples was verified by the apparently identical intensities of 18S rRNA bands with ethidium bromide staining. Mouse tissue extracts (80 μg each) were subjected to Western blot analysis using antiserum against human Tom34 (3) at 1:1,000 dilution. Polypeptides were visualized using an enhanced chemiluminescence kit (Amersham Bioscience). (B) Expression of Tom34 mRNAs containing exon 1a or exon 1b. Total RNA samples prepared from indicated tissues were reverse-transcribed and amplified by PCR using specific forward primers located in exon 1a (primer 1a) and exon 1b (primer 1b) as indicated in Fig. 1B. A reverse primer located in exon 4 (primer 2) was used for both amplifications. Sizes of amplified fragments containing exon 1a and exon 1b were 548 and 596 bp, respectively.

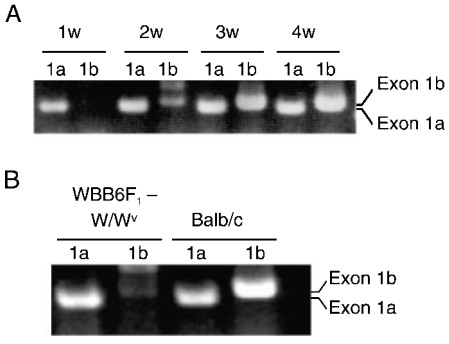

Fig. 3. **Expression of Tom34 exon 1a and 1b in testes from wild-type BALB/c mice at different developmental stages (A) and from adult wild-type BALB/c and germ cell-deficient mice (B).** (A) Total testes RNA samples from Balb/c mice (1, 2, 3, and 4 week *post partum*) were analyzed by RT-PCR to detect expression of exon 1a and exon 1b. (B) Total testes RNA samples from germ cell-deficient WBB6F$_1$-W/W^v mice (8 weeks) and BALB/c mice (8 weeks) were analyzed by RT-PCR to detect expression of exon 1a and exon 1b.

was hardly detected from testes at 1 week *post partum*, but its expression gradually increased from 2 to 4 weeks *post partum*. The Tom34a mRNA was also detected and did not change during the period. We next examined expression of the two mRNAs in testis from a germ-cell-deficient mouse, WBB6F$_1$-W/W^v (*28*, *29*). Both mRNAs were readily detected in testes from wild-type BALB/c

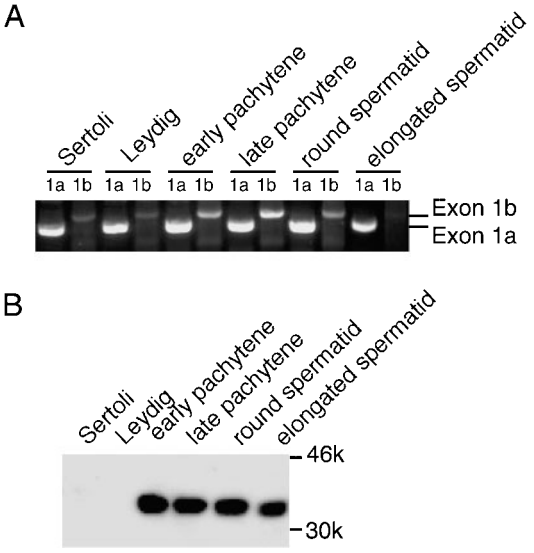

Fig. 4. **Expression of Tom34 in fractionated germ cells.** (A) Total RNA samples from fractionated germ cells were analyzed by RT-PCR to detect expression of exon 1a and 1b. Annealing temperature for the PCR amplification was more stringent than that applied for Figures 2 and 3. (B) Cell extracts from fractionated germ and somatic cells (10 μg protein each) were subjected to Western blot analysis using antiserum against human Tom34 at 1:1,000 dilution.

mice. However, only Tom34a mRNA was detected in WBB6F$_1$-W/W^v mutant mice (Fig. 3B). These results suggest that the mouse Tom34b mRNA is specifically expressed in male gonadal germ cells and that its expression increases during germ cell maturation.

Expression of Tom34 in Fractionated Testicular Cells—Expression of Tom34 mRNAs in fractionated germ cells was analyzed using RT-PCR (Fig. 4A). Tom34a mRNA was detected in all testicular cells. On the other hand, Tom34b mRNA was highly expressed in early pachytene to late pachytene cells. However, its expression was decreased significantly in round spermatid cells.

Figure 4B shows Western blot analysis of Tom34 proteins. We used an antiserum against human Tom34 that lacks the NH$_2$-terminal hydrophobic region (*15*); the antigen corresponds to the mouse Tom34 product starting from exon 3. The antiserum crossreacted with mouse Tom34 protein. Tom34 protein was readily detected in all testicular germ cell stages and mature adult spermatid cells. In contrast, Tom34 protein was hardly detected in testicular somatic cells, such as Sertoli and Leydig cells. The level of Tom34 protein in various testicular cells correlated with that of Tom34b mRNA.

Generation of Tom34-Deficient Mice—To better understand the biological significance of Tom34 in mouse, we disrupted the *Tom34* gene. A targeting vector was constructed by inserting the neomycin phosphotrasferase (*neo*) gene between exon 1a and exon 1b of the *Tom34* gene. The diphtheria toxin A fragment (DT-A) gene was also included in the targeting vector for negative selection of homologous recombinants (Fig. 5A). The vector was introduced into the TT2 embryonic stem cells, and 14 homologous recombinant clones were identified by PCR among 72 G418-resistant clones. Their genotype was con-

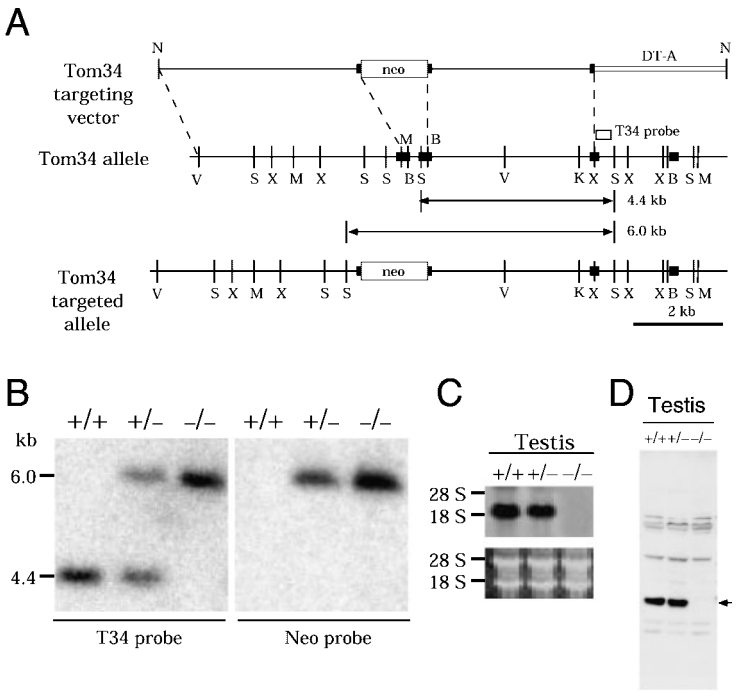

Fig. 5. **Generation of Tom34-deficient mouse.** (A) Targeting vectors for *Tom34* mutation. Exons 1a, 1b, 2, and 3 are indicated by filled rectangles. The targeting vector contains a neomycin resistance gene (*neo*) for positive selection and the diphtheria toxin A gene (DT-A). The predicted sizes of normal and targeted alleles and the location of T34 probe used in Southern blot analysis (B) are shown. B, *Bss*HII; K, *Kpn*I; M, *Msc*I; N, *Not*I; S, *Sac*I; V, *Eco*RV; X, *Xmn*I. (B) Examples of Southern blot analyses by *Sac*I digestion of genomic DNAs from male mice using T34 probe (left panel) and *neo* probe (right panel). (C) Northern blot analysis of Tom34 from testes. Total RNA samples were analyzed using digoxigenin-labeled RNA as a probe (upper panel). Integrity of the RNA samples was verified by the apparently identical intensities of 18S and 28S rRNA bands with ethidium bromide staining (lower panel). (D) Western blot analysis of Tom34 from testes. Mouse tissue extracts (30 μg each) were subjected to Western blot analysis using antiserum against human Tom34 at 1:1,000 dilution. An arrow indicates the position of Tom34.

firmed by Southern blot analysis. Two independent homologous recombinant ES clones were selected and injected into ICR 8-cell-stage embryos to generate chimeric mice. Male chimeras were mated with C57BL/6 females to generate *Tom34*$^{+/-}$ mice. The *Tom34*$^{+/-}$ mice were obtained from two independent mutant ES clones and intercrossed to generate *Tom34*$^{-/-}$ mice. Southern blot analysis confirmed the genotype of the *Tom34*$^{-/-}$, *Tom34*$^{+/-}$ and wild-type mice (Fig. 5B). *Tom34*$^{-/-}$ mice were born at the expected Mendelian ratio and did not show any gross abnormality up to one year in two mouse strains derived from independent ES clones.

Development and Function of Male Germ Cells in Tom34-Deficient Mice—Northern blot analysis revealed the absence of any Tom34 transcripts in adult *Tom34*$^{-/-}$ testes (Fig. 5C). Neither form of Tom34 protein was detected (Fig. 5D). Since Tom34 protein is highly expressed in testicular germ cells, we initially performed histochemical analysis of testes. As shown in Fig. 6, spermatogenesis in *Tom34*$^{-/-}$ testes was normal. Immunohistochemical analysis of testes from wild-type and *Tom34*$^{-/-}$ mice showed abundant expression of Tom34 proteins in pachytene cells in wild-type testis, the localization of which accords well with the expression pattern in the fractionated testicular cells (Fig. 4B). No significant difference was observed with regard to size and weight of testes among the age-matched wild-type, *Tom34*$^{+/-}$ and *Tom34*$^{-/-}$ littermates (data not shown). Furthermore, the effect of Tom34 deficiency on sperm maturation was assessed by monitoring mature sperms from the epididymis. Again, no apparent difference was observed with regard to number and motility of sperm between wild-type and *Tom34*$^{-/-}$ mice (data not shown). The presence of viable sperm indicates that Tom34 is not essential for spermatogenesis.

Next we examined the fertilizing ability of male *Tom34*$^{-/-}$ mice. As shown in Table 1, *Tom34*$^{-/-}$ male X *Tom34*$^{-/-}$ female breeding pairs were fertile and the average litter size was similar to that of wild-type pairs. In conclusion, the absence of Tom34 does not affect the fertility in mice.

In Vitro Protein Import into Mitochondria from Tom34-Deficient Mice—Since the soluble domain of Tom34 and antibodies against it strongly inhibit *in vitro* mitochondrial import of several preproteins (*15*), the absence of Tom34 on the outer membrane of mitochondria may affect preprotein import. To test this, we performed *in vitro*–import of pre-ornithine transcarbamylase into the mitochondria isolated from testes of Tom34$^{-/-}$ mice (Fig. 7). However, mitochondria from Tom34$^{-/-}$ mice showed a similar import activity to mitochondria from wild-type mice. In accord with this observation, the content of liver ornithine transcarbamylase showed no difference between age- and sex-matched *Tom34*$^{-/-}$ and wild-type mice (data not shown).

Table 1. **Offsprings of Tom34-deficient mouse.**

Tom34-genotype	No. of litters	Litter size*
Female (*Tom34*$^{+/+}$) × Male (*Tom34*$^{+/+}$)	(n = 11)	7.0 ± 2.4
Female (*Tom34*$^{-/-}$) × Male (*Tom34*$^{-/-}$)	(n = 20)	7.2 ± 3.1

Appropriate genotypes of adult mice (3 months old) were mated for 2 months. *Mean ± SD.

DISCUSSION

The mouse *Tom34* gene is transcribed to produce two species of mRNA. By using an RT-PCR technique, we demonstrated that one has exon 1a and the other has exon 1b. However, the expected sizes of these two mRNAs are very similar, and we could not resolve them by Northern blot analysis. The expression levels of these two mRNAs are different among tissues. Tom34b mRNA is expressed most strongly in the pachytene stage of testicular germ

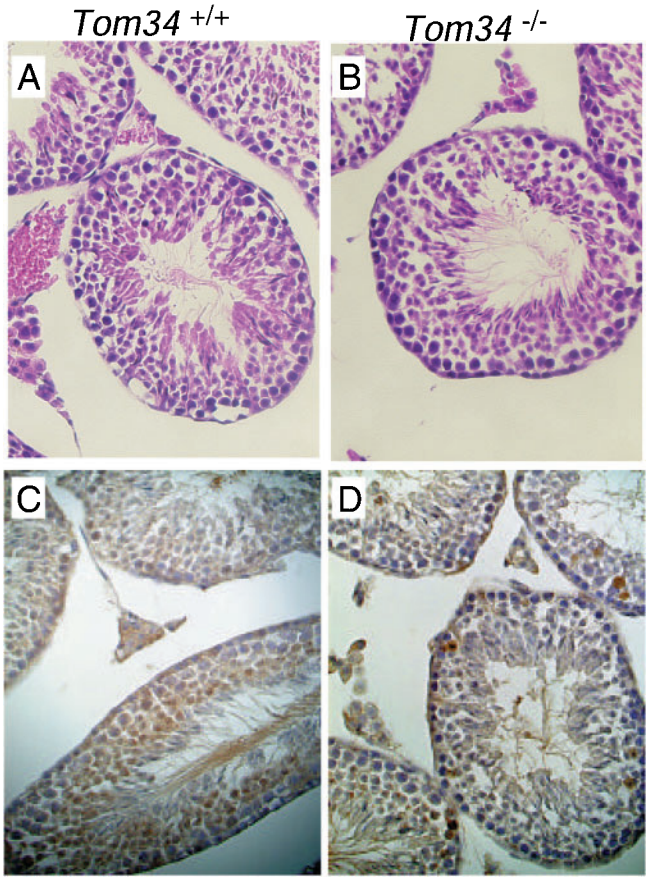

Fig. 6. **Histology of Tom34-deficient testes.** Hematoxylin-eosin-stained formalin-fixed paraffin sections (5 μm) of wild-type (A) and *Tom34*-deficient (B) testes. Imunostaining of Tom34 in wild-type (C) and *Tom34*-deficient (D) testes. Antiserum against Tom34 (1:100) was used as the primary antibody. Peroxidase activity was visualized using 3, 3′-diaminobenzidine as a substrate. Sections were counterstained with hematoxylin.

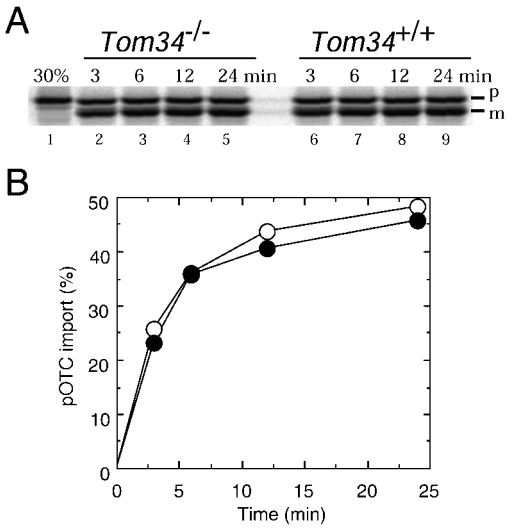

Fig. 7. ***In vitro* import of pre-ornithine transcarbamylase into testes mitochondria from wild-type and *Tom34*-deficient mice.** Rabbit reticulocyte lysate (4 μl) containing ^{35}S-labeled rat pre-ornithine transcarbamylase was imported into mitochondria isolated from wild-type (open circle) or *Tom34*-deficient (filled circle) mouse testis. A portion of the imaging plate picture (A) and the quantitated results (B) are shown. p, preprotein; m, mature protein. 30%, 30% of the input preprotein. pOTC, pre-ornithine transcarbamylase.

cells. The predicted amino acid sequences of the two *Tom34* proteins are 99% identical and their numbers of residues are equal. Within the 42 residues encoded by exon 1a and exon 1b, only four residues are different. Thus, we could not resolve the two Tom34 proteins by Western blot analysis. The expression level of Tom34b mRNA correlated well with that of Tom34 protein in fractionated testicular cells. In contrast, Tom34a mRNA was expressed ubiquitously in testicular cells, suggesting translational control. Interestingly, an 8-bp stem hairpin with a 16-nt loop exists in the 5′ untranslated region of exon 1a (Fig. 1B). A hairpin structure adjacent to the initial codon may affect translational efficiency. The significance of the hairpin structure in exon 1a for translation of Tom34a mRNA remains to be elucidated.

To investigate the *in vivo* function of Tom34, especially in testis, we generated *Tom34*-deficient mice. The *Tom34*$^{-/-}$ mice showed no obvious abnormal phenotype in terms of growth, macroscopic anatomy, behavior, fertility, and biochemical parameters of the serum (data not shown). Therefore, Tom34 is dispensable for development and growth of mouse under environmentally optimal conditions. However, the possibility remains that Tom34 is necessary under non-optimal conditions such as stressed conditions.

Although the *Tom34*$^{-/-}$ mouse was expected to have some defect in mitochondrial protein import, we observed no difference in *in vitro* import of pre-ornithine transcarbamylase into mitochondria from wild-type and *Tom34*$^{-/-}$ mice. Tom34 contains a copy of the TPR domain found in Tom70 (*30*). The Tom70 receptor mainly recognizes proteins with internal targeting sequences, such as the metabolite carriers of the inner mitochondrial membranes (*31*). If the TPR domain of Tom34 interacts with those of Tom70, the absence of the Tom34 may influence mitochondrial import of the Tom70-dependent passenger proteins. The failure of transport of metabolites across the inner membrane by the metabolite carriers is expected to be deleterious to the animal. However, we found no difference in viability between the wild-type and the *Tom34*$^{-/-}$ mice. Thus, we conclude that mitochondria-associated Tom34 is a dispensable factor in mitochondrial protein import under normal conditions.

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