MOLECULAR AND CELLULAR BIOLOGY, Feb. 2004, p. 1168–1173 Vol. 24, No. 3
0270-7306/04/$08.00+0 DOI: 10.1128/MCB.24.3.1168–1173.2004

More than blood, a Novel Gene Required for Mammalian Postimplantation Development

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Received 11 September 2003/Accepted 30 October 2003

***More than blood* (*Mtb*) is a novel gene that is widely expressed in mouse embryos prior to gastrulation but is subsequently restricted to specific tissues, including the developing central nervous system and hematopoietic organs. Since MTB is highly expressed in the fetal liver and developing thymus, we predicted that MTB would be required for hematopoiesis and that embryos deficient in MTB would die of anemia. Surprisingly, embryos with a targeted disruption of *Mtb* died prior to the initiation of blood cell development, immediately following implantation. This lethality is due to a defect in expansion of the inner cell mass (ICM), as $Mtb^{-/-}$ blastocysts failed to exhibit outgrowth of the ICM, both in vitro and in vivo. Furthermore, $Mtb^{-/-}$ blastocysts exhibited a higher frequency of apoptotic cells than wild-type or heterozygous blastocysts. These findings demonstrate that *Mtb* is a novel gene that is essential for early embryonic development.**

Gene targeting studies have highlighted the importance of many genes in preimplantation and immediate postimplantation development of the mouse embryo. Early embryonic development requires proteins that are known to be involved in a wide array of cellular processes, including growth factor signaling, cell adhesion, cell cycle regulation, DNA replication and repair, chromatin modifications, and transcription. Fibroblast growth factor 4, which is expressed by the inner cell mass (ICM), and its receptor, fibroblast growth factor receptor 2, are both required for proper expansion of the blastocyst upon implantation (1, 6). E-cadherin and β1 integrin, two proteins involved in cell adhesion, are required for preimplantation and postimplantation development, respectively (11, 19, 25). Deletion of cell cycle regulators, such as cyclin A2 or cdc7, also results in peri-implantation embryonic lethality (9, 14). Several proteins involved in DNA replication and repair, including cdc45, a putative DNA polymerase loading factor (29), members of the ATR/ATM superfamily of cell cycle checkpoint genes (2, 4), Chk1 (12), and Rad51 (26), are necessary for pregastrulation development of the mouse embryo. Chromatin maintenance and modifying activities, such as components of the SWI/SNF complex (7, 10) and *polycomb*-related proteins (16), also play a critical role in early embryogenesis. Finally, various proteins involved in transcription, from basal transcription factors such as TFIIH (3, 21) to ubiquitous transcription factors such as YY1 (5) to lineage-restricted regulators such as Oct-4 (15), are also essential for early embryonic development in the mouse. A major challenge in analyzing these early developmental defects is distinguishing those genes that are essential for basic cellular viability from those that are specifically required for a developmental program.

One of the best-studied regulators of early mammalian development is the POU domain transcription factor Oct-4 mentioned above. In the early embryo, Oct-4 functions at the first differentiation event in determining whether a cell is destined to become part of the trophectoderm or the ICM, which generate the extraembryonic tissues, including the placenta, or the embryo proper, respectively (for a review, see reference 17). In the absence of Oct-4, all blastomeres become trophoblasts, and the embryo dies shortly after implantation (15). The requirement for Oct-4 in the specification of embryo-derived stem cells is unique, because Oct-4 is not ubiquitously expressed, nor does it play a role in general cellular function.

While Oct-4 is not known to regulate somatic stem cells, transcription factors of the basic helix-loop-helix (bHLH) family are involved in lineage specification in multiple tissues, including blood, brain, and muscle. Class I bHLH proteins, which include the products of the E2A gene (E12 and E47), HEB, and E2-2, form homo- and/or heterodimers that bind DNA of the consensus site CANNTG (for a review, see reference 13). Although these proteins are ubiquitously expressed, gene targeting studies with mice have uncovered specific roles for these factors in B- and T-cell development (18). The absence of more widespread deficiencies in these single-gene-targeted mice indicates that the family members exhibit redundant roles in nonlymphoid lineages (30). In contrast, the class II bHLH factors are expressed in a tissue-specific manner. These factors typically interact with DNA as heterodimers with E-proteins and activate transcription of lineage-specific genes. Essential class II proteins include the muscle factors myogenin and myoD, the neural factor MASH-1, and the hematopoietic protein SCL/tal-1. SCL is required for embryonic specification of hematopoietic stem cells, as mice lacking SCL die in utero between embryonic day 8.5 (E8.5) and E10.5 due to a complete absence of blood cells (20, 24).

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In this study, we describe the cloning and targeted deletion of a novel gene named *More than blood* (*Mtb*). *Mtb* is widely expressed in mouse embryos prior to gastrulation but then is expressed primarily at sites of neurogenesis and hematopoiesis in embryos at mid- to late gestation and in adult mice. Although MTB was identified in a yeast two-hybrid screen for SCL-interacting proteins, the physiological relevance of this putative association has not been established. It is clear, however, that MTB is required for early embryonic development and is essential for viability and expansion of the ICM of the implanting blastocyst. *Mtb* joins an expanding list of genes whose activity is required at early stages of mammalian development.

MATERIALS AND METHODS

Yeast two-hybrid screen. Full-length SCL cDNA was fused in frame to the DNA binding domain of Gal4 in the pGBT9 vector (Clontech). The library contained cDNAs from MEL cells fused in frame to the Gal4 activation domain (AD) in plasmid pGADGL (Clontech). MaV103 yeast cells (27) were cotransformed with the bait and prey by standard methods. Clones harboring interacting proteins were selected on SD medium lacking Leu, Trp, and His and with 10 mM 3-amino-1,2,4-triazole. Positive clones were restreaked to SD medium lacking Leu, Trp, and Ura to determine whether the interaction could drive a second reporter, Ura3. Cells were also analyzed for LacZ expression by standard filter assays. DNAs from 10 positive clones were isolated by standard methods and then transfected into bacteria by electroporation. The library plasmids were identified and sequenced to reveal the interacting gene. Of the 10 clones that activated both reporters, 6 harbored DNAs encoding the class A bHLH protein ALF-1 (mouse homologue of HEB), 1 encoded murine transcription factor A1 (a leucine zipper-bHLH protein), and the final 3 clones contained identical fragments of MTB.

Cloning of full-length *Mtb*. The MTB fragment obtained from the yeast two-hybrid screen (named AD4) was approximately 650 bp in length and was predicted to encode 211 amino acids. We probed a murine cDNA phage library with this MTB fragment, according to standard methods (23), and identified two overlapping phage clones. In addition, we performed both 5′ and 3′ rapid amplification of cDNA ends (RACE), using the SMART RACE cDNA amplification kit (Clontech). The complete *Mtb* coding sequence is 2.5 kb and is predicted to encode a polypeptide of 849 amino acids.

Northern blots and in situ expression analysis. Total RNA or poly(A)$^+$ RNA was isolated from various murine cell lines or tissues by using Trizol reagent (Invitrogen). The remaining Northern blots were purchased from Clontech. Northern blots were hybridized with various portions of radiolabeled MTB cDNA. For in situ hybridization, an antisense RNA probe corresponding to the MTB AD4 fragment or an antisense SCL RNA was prepared by using digoxigenin-UTP (Boehringer Mannheim), and in situ hybridization was performed as described previously (28).

Gene targeting and genotyping. The targeting vector was constructed in pBluescript KS(−) with herpes simplex virus thymidine kinase inserted into the *Sal*I site and LoxP-neomycin inserted into the *Not*I site, with flanking by a 10-kb *Sac*I-*Xba*I genomic fragment for the 5′ homology region and a 4-kb *Bam*HI-*Spe*I genomic fragment for the 3′ homology region. Upon homologous recombination, the neomycin cassette replaces bp 937 to 2293 of the 2.5-kb coding region. The targeting construct was electroporated into CJ7/129 embryonic stem cells and selected with 280 μg of neomycin per ml. Genomic DNA was isolated from neomycin-resistant clones, digested with *Sac*I, and genotyped by Southern blotting with a 200-bp probe (bp 2298 to 2498 of the coding region). One *Mtb*$^{+/-}$ clone with a normal diploid number was identified from approximately 500 clones screened and was used for subsequent injection into C57BL/6 blastocysts. The injected blastocysts were then implanted into pseudopregnant females to generate chimeric mice. *Mtb* chimeric males were crossed with wild-type C57BL/6 females to generate germ line *Mtb*$^{+/-}$ progeny. The first 10 litters were genotyped by Southern blot analysis as described above, and the subsequent litters and embryos were genotyped by single-reaction PCR with the primers Wt1For (5′ TTCATCACCACCATCCAG 3′), Neo1For (5′ CTCCAGACTGC CTTGGGAAAAG 3′), and Com3Rev (5′ CAGAGCAGTCCTGAATAG TCTG 3′) to generate products of 434 bp for the wild-type allele and 616 bp for the targeted allele. PCR genotyping reactions were performed in mixtures containing 1× buffer 6 (Stratagene), 200 μM deoxynucleoside triphosphates, a 1 μM concentration of each forward primer, a 2 μM concentration of the common reverse primer, 10% dimethyl sulfoxide, and 1 μl of Klentaq DNA polymerase (Clontech) with the following program: 94°C for 3 min; 30 cycles of 94°C for 45 s, 60°C for 1 min, and 72°C for 1 min; and a final 10-min extension at 72°C.

Embryo isolation and culture. To determine the stage of embryonic lethality, timed matings were established between *Mtb*$^{+/-}$ males and females. Noon of the day a vaginal plug was detected was defined as 0.5 day postcoitum. Embryos from E3.5 to E12.5 were isolated essentially as previously described (8). For blastocyst cultures, blastocysts were flushed from dissected uterine horns in M2 medium (Sigma), and zona pellucidae were removed by pronase treatment (8). Blastocysts were cultured on gelatinized embryo dishes (Becton Dickinson) in embryonic stem cell medium (high-glucose Dulbecco's modified Eagle's medium [Invitrogen])–15% fetal calf serum (HyClone)–penicillin-streptomycin–200 mM L-glutamine–1% nucleoside mix plus leukemia inhibitory factor (LIF) (1,000 U/ml) for 3 to 6 days. Following the growth period, cultured blastocysts were harvested by trypsinization, resuspended in 10 μl of embryo lysis buffer (1% Triton X-100, 50 mM Tris [pH 8], 20 mM NaCl, 1 mM EDTA) with 100 ng of proteinase K per μl, and digested overnight at 55°C. Following heat inactivation of proteinase K, 5 μl of embryo lysate was genotyped by nested PCR. In the first round, primers Wt1For, Neo2For (5′ CAAAGCTGCTATTGGCCGCTG 3′), and Com3Rev were used for 30 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 90 s. This amplification reaction generates a wild-type product of 434 bp and a mutant product of 905 bp. One microliter of this 50-μl PCR product was used as the template for the second round of PCR with primers Wt1For, Neo1For, and Com2Rev (5′ GGAGTGATGGCATCC TCAGCAC 3′), with a program of 30 cycles of 94°C for 45 s, 60°C for 60 s, and 72°C for 60 s. The final products are 285 bp (wild-type allele) and 466 bp (mutant allele).

TUNEL assays. Terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) assays were performed on freshly isolated blastocysts from *Mtb* heterozygous matings at E3.5. The blastocysts were fixed in freshly prepared 4% paraformaldehyde in phosphate-buffered saline (PBS) and washed in PBS with 5% bovine serum albumin, and TUNEL reactions were performed with an in situ cell death detection kit (fluorescein label) (Roche). Blastocysts were stained with 1 μg of Hoechst 33342 (Intergen) per ml to detect nuclei. TUNEL positivity was detected by fluorescence microscopy with a Zeiss Axiovert 100TV microscope, a Micromax digital camera, and Slidebook 3.0 and Openlab 3.1 imaging software (University of Chicago Cancer Research Center Digital Light Microscopy Facility). TUNEL-positive nuclei were quantitated by performing a digital Z stack through the blastocyst. Following imaging, blastocysts were washed in PBS with 5% bovine serum albumin, transferred to 10 μl of embryo lysis buffer, and processed for genotyping by nested PCR as described above.

Nucleotide sequence accession number. The MTB cDNA sequence has been deposited in GenBank under accession number AY455829.

RESULTS AND DISCUSSION

Cloning of *Mtb*. MTB was isolated in a yeast two-hybrid screen for SCL-interacting proteins. The *Mtb* fragment obtained from the yeast two-hybrid screen comprised the extreme C-terminal domain of the protein (residues 639 to 849, named the AD4 fragment). To clone the entire cDNA, we screened a phage library with the AD4 fragment and additionally performed both 5′ and 3′ RACE. The complete *Mtb* cDNA is 6.8 kb and is predicted to encode a protein of 849 amino acids with little similarity to known proteins. MTB contains weak homology to a leucine zipper motif and harbors several putative casein kinase II and protein kinase C phosphorylation sites (Fig. 1 and data not shown). Database searches revealed that *Mtb* is a novel murine gene with a human homologue, a predicted gene named FLJ20311. Murine *Mtb* and human FLJ20311 are highly homologous at both the DNA (87% identity) and protein (80% identity) levels.

MTB is initially widely expressed in embryos but then becomes enriched in sites of hematopoiesis and neurogenesis in the developing embryo and adult mice. We performed both in situ hybridization of mouse embryos and Northern blot analyses to determine the expression profile of MTB. Two tran-

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  1 MFHGIHLLRR SPVHSKVREV LSYFHQQKVR QGVEEMLYRL YKPILWRGLK
 51 ARNSEVRSNA ALLFVEAFPI RDPNFTATEM DNEIQKQFEE LYNLIEDPYP
101 RVRSTGILGV CKISSKYWEM MPPNILVDFL KKVTGELAFD ISSADVRCSV
151 FKCLPIILDN KLSHPLLEQL LPTLRYSLHD NSEKVRVAFV DLLLKIKAVR
201 AAKFWKICPM EDILVRLEMD SRPVSRRLVS LIFNSFLPVN QPEEVWCERC
251 VTLIQMNRAA ARRFYQYAHE HTASTNIAKL IHVIRHCLNA CIQRTLREGP
301 EAHKECEKEN ASVLDKTLSV NDTASMAGLL EIIVILWKNI HRSLENNKEA
351 KIYTINKFAA VLPEYLKVFK DERCKIPLFM LMSFLPASAV PVFSCGVISV
401 LRNQESVTGR SYCTLLDCLC SWGQVGHVLE LIVGWLPTVP PQAKSNLASK
451 RKVEINDTCS VKPELALLCM EYLLTHPKNR ECLLSVPQKK LNQLLKALEG
501 SKAELESFLQ SPSGNPLNFN KATALHAFGL YCRMSVHLQY KFCSEEKIHL
551 SILDDTGSWL ENKVLPLLED QEEEYLKLRK DVYQQIIQTY LAVCKDVVMV
601 GLGDPKFQMQ LLQRSFGIMK TVKGFFYVSL LLGILKEIAG NTIIHKTDSD
651 EKVTVLFDLV QEVFQKMLEC IACIFRKQPE ESLPLFHSVQ TPLHEFITTI
701 QSWHKDTAVH HAVLSTLIAA PVVEISHQLQ KVSDIEELTS PQCLHDLPPF
751 SRCLVGVIMK SSDVVRSFVD ELKACVTSGD VEGIVCLTAV LHIILVINKG
801 KHISAKVKEV AAAVYRKLKT FMEITLEEDS LERFLYESSM RTLGEFLNP
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FIG. 1. Amino acid sequence of murine MTB. The region with homology to a leucine zipper domain is shaded.

scripts of MTB were detected in a Northern blot of RNA from embryos as early as E7.0, the stage when yolk sac hematopoiesis is initiated (Fig. 2A). These two transcripts differ in the 5′ and 3′ untranslated regions. Whole-mount in situ hybridization demonstrated that MTB is widely expressed in embryos at both E6.5 and E8.5 (Fig. 3A and data not shown). However, MTB expression is subsequently restricted to specific regions of the embryo, including the developing fetal liver, the site of definitive hematopoiesis, at both E12.5 and E15.5 (Fig. 3B and C). In addition, MTB expression is detected in the thymus at E15.5, as well as in the midbrain, forebrain, and the olfactory epithelium (Fig. 3C, D, and E). In adult mice, MTB is expressed in the spleen, lung, and testis, as well as in all hematopoietic cell lines tested, including megakaryocytes and erythroid, myeloid, and lymphoid cells (Fig. 2B and C and data not shown). Expression was not detected in NIH 3T3 cells or adult muscle tissue (data not shown). Together, these expression data illustrate that MTB exhibits a broad expression profile in early embryos and that MTB is later enriched in hematopoietic and neuronal lineages.

Disruption of the *Mtb* locus results in embryonic lethality. In order to examine the role of MTB in vivo, we disrupted the *Mtb* locus in embryonic stem cells (Fig. 4A) and subsequently injected $Mtb^{+/-}$ embryonic stem cells into wild-type blastocysts to generate chimeric mice. Chimeric males were mated to wild-type females to generate germ line $Mtb^{+/-}$ mice. Southern and PCR genotyping strategies were used to identify mutant mice (Fig. 4B and C). *Mtb* heterozygous mice were normal at birth, grew at a rate similar to that of their wild-type littermates, and were fertile. The $Mtb^{+/-}$ males and females were bred to generate $Mtb^{-/-}$ progeny. After genotyping over 30 litters from heterozygous matings, no $Mtb^{-/-}$ pups were identified, indicating that disruption of the *Mtb* locus resulted in embryonic lethality (Table 1). Genotyping of embryos from E6.5 to E12.5 demonstrated that $Mtb^{-/-}$ embryos died prior to blood cell development and even before gastrulation (Table 1). Furthermore, approximately 25% of the embryos analyzed between E6.5 and E12.5 were resorbed (Table 1). This high frequency of resorption suggested that *Mtb*-deficient embryos implant normally but then fail to develop. To confirm this, we analyzed embryos in stained uterine sections at E5.5 (Fig. 5A and B). While the majority of embryos at this stage have generated an egg cylinder (Fig. 5A), approximately 25% of the

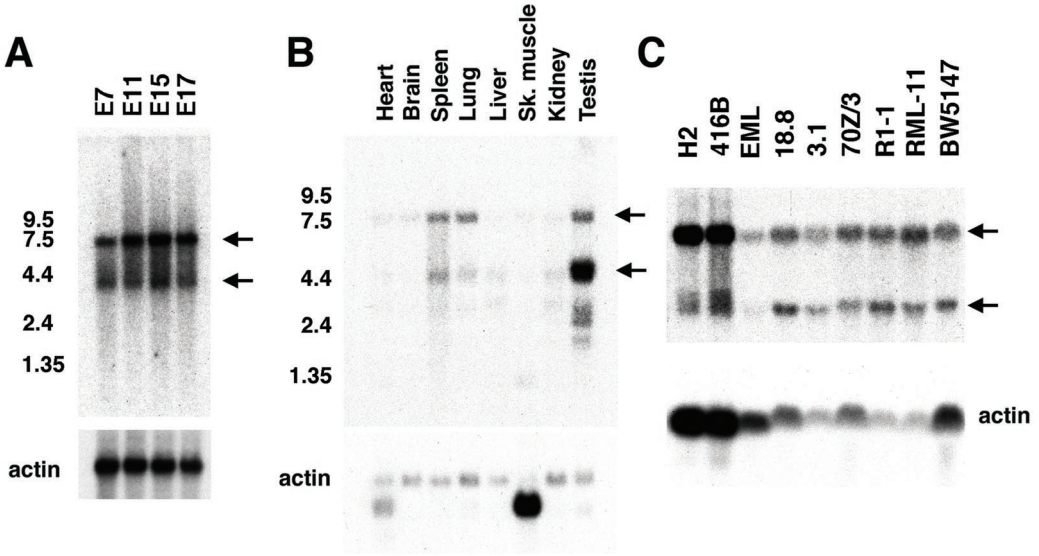

FIG. 2. Northern blot analysis of MTB expression during embryogenesis, in adult tissues, and in hematopoietic cell lines. (A) MTB expression can be detected as early as E7.0, as revealed by hybridization of a Clontech embryonic tissue blot with the C-terminal AD4 domain of MTB. Two primary forms of MTB, of approximately 6.8 and 4.4 kb, exist (arrows). These two transcripts differ in the 5′ and 3′ untranslated regions (data not shown). (B) Hybridization of a Clontech multiple tissue blot with the MTB probe revealed that MTB expression is restricted to the spleen, lung, and testis of adult mice. (C) MTB is expressed in megakaryocytic cells (H2), myeloid cells (416B), EML cells, and a variety of pre- and pro-B-cell lines (18.8, 3.1, and 70Z/3), as well as in multiple T-cell lines (R1-1, RML11, and BW5147). The first two lanes contain poly(A)$^+$ RNA, while the other lanes contain total RNA. Hybridization of all three blots with actin is shown below the MTB blots. MTB is also expressed in the erythroid cell lines MEL and G1E (data not shown).

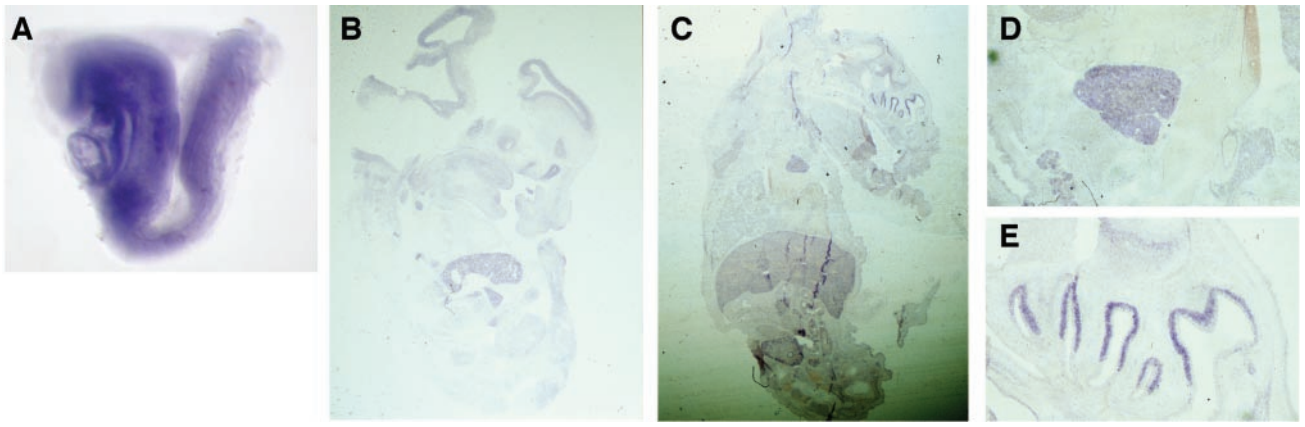

FIG. 3. MTB expression in early- and mid-gestation embryos. (A) Whole-mount in situ hybridization, using the AD4 probe, of a wild-type embryo at E8.5 highlights the broad expression pattern of MTB at this early stage of development. (B) In situ hybridization of a mid-sagittal section of a wild-type embryo at E12.5 with the AD4 probe. Note the intense staining of the fetal liver, forebrain, and midbrain. (C, D, and E) In situ hybridization of an E15.5 mid-sagittal embryo section with the MTB probe. MTB expression is abundant in the fetal liver (C), thymus (D), and olfactory epithelium (E).

embryos were developmentally delayed (Fig. 5B). These findings support the conclusion that *Mtb*-deficient embryos implant but fail to undergo the proliferative burst that normally leads to the formation of the egg cylinder. This analysis demonstrates that MTB is required for postimplantation development.

***Mtb* is required for ICM and embryonic stem cell growth.** To further characterize the growth defect of $Mtb^{-/-}$ embryos, we isolated blastocysts from the uteri of pregnant $Mtb^{+/-}$ females at E3.5, prior to implantation. Wild-type and $Mtb^{-/-}$ blastocysts were indistinguishable upon isolation. Blastocysts were then cultured on gelatinized tissue culture dishes in embryonic stem cell medium containing LIF for 3 to 6 days and subsequently genotyped by PCR. All blastocysts attached, and trophoblasts adhered to the culture dish normally. The ICMs of both wild-type and $Mtb^{+/-}$ embryos formed a large outgrowth

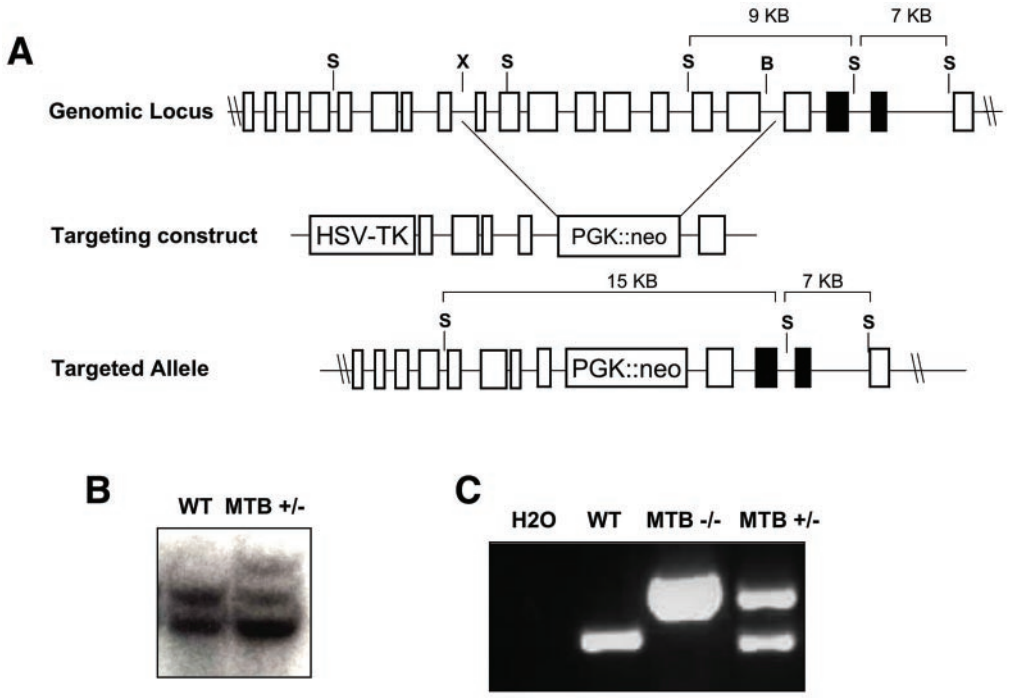

FIG. 4. Targeted disruption of *Mtb*. (A) Targeting strategy for the *Mtb* locus, replacing approximately 1.3 kb of coding sequence in the middle of the locus with the neomycin cassette. Open boxes indicate known coding exons, and shaded boxes indicate the probe sequence used for Southern hybridization. S, *Sac*I; X, *Xba*I; B, *Bam*HI. (B) Southern blot analysis of genomic DNA isolated from tail clippings and digested with *Sac*I. The wild-type (WT) allele gives rise to 9- and 7-kb fragments, while digestion of the mutant allele generates 15- and 7-kb fragments. (C) Single-reaction PCR genotyping analysis with no template DNA, genomic DNA (for wild-type and heterozygous reactions), or the targeting construct in the case of the null. The wild-type band is 434 bp, and the mutant band is 616 bp.

TABLE 1. Disruption of *Mtb* results in embryonic lethality prior to gastrulation

Mice	No.				
	$Mtb^{+/+}$	$Mtb^{+/-}$	$Mtb^{-/-}$	Resorbed	Total
Live-born pups	84	162	0	NA[a]	246
Embryos					
E9.5–E12.5	5	23	0	8	36
E8.5	10	14	0	10	34
E7.5	16	25	0	18	59
E6.5	14	17	0	5	36
Total	45	79	0	41	165

[a] NA, not applicable.

after several days in culture, whereas the $Mtb^{-/-}$ blastocysts failed to generate an ICM outgrowth (Fig. 5C and D). Together, these data demonstrate that MTB is necessary for the proliferative burst of the ICM both in vivo (Fig. 5A and B) and in vitro (Fig. 5C and D). Furthermore, these results indicate that MTB is essential for growth of ICM stem cells, the embryonic population from which pluripotent embryonic stem cells are derived. An intrinsic requirement for MTB in embryo-derived stem cells is also supported by our inability to generate $Mtb^{-/-}$ embryonic stem cell lines by multiple methods, including neomycin step-up protocols, retargeting of the wild-type allele, and blastocyst culturing (data not shown). Consistent with this requirement for MTB in early embryonic development, MTB transcripts were detected in embryonic stem cells by reverse transcription-PCR (data not shown).

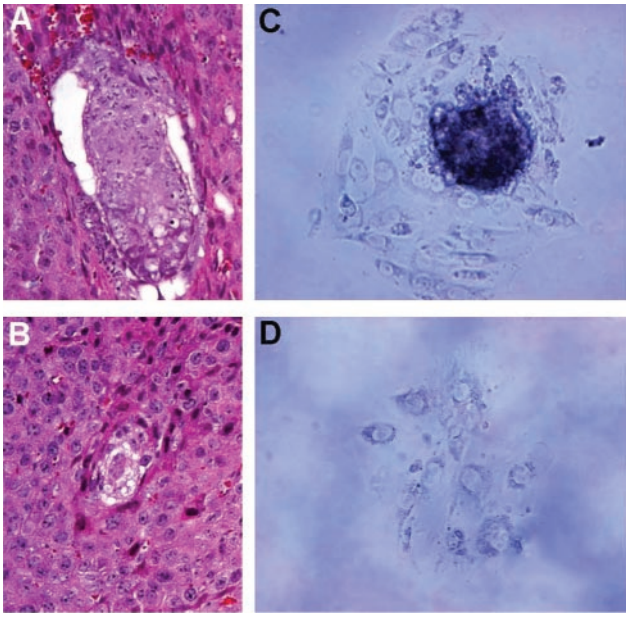

FIG. 5. $Mtb^{-/-}$ embryos fail to undergo a proliferative burst in vivo and in vitro. (A and B) Hematoxylin- and eosin-stained sections of mouse uterus containing presumed wild-type (A) and $Mtb^{-/-}$ (B) embryos at E5.5. (C and D) Blastocyst outgrowths of wild-type (C) and $Mtb^{-/-}$ (D) blastocysts isolated from a uterine flush, cultured for 6 days on gelatinized dishes in embryonic stem cell medium, and genotyped by nested PCR. The ICM of $Mtb^{-/-}$ embryos does not expand in the uterus or in culture.

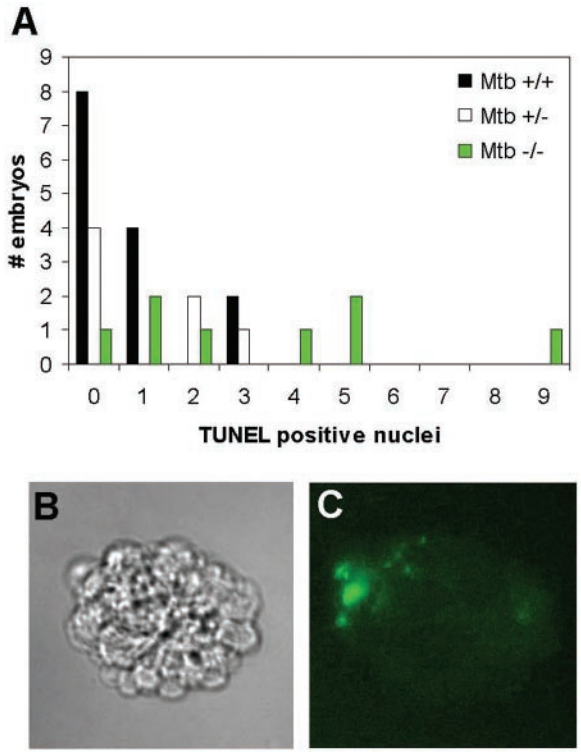

FIG. 6. Increased incidence of apoptosis in $Mtb^{-/-}$ blastocysts. Blastocysts were isolated at E3.5, fixed, and subjected to TUNEL. (A) Quantitation of TUNEL positivity was performed by fluorescence microscopy (see Materials and Methods). $Mtb^{-/-}$ blastocysts have a significantly higher number of TUNEL-positive nuclei than their wild-type ($P < 0.001$) or $Mtb^{+/-}$ ($P < 0.05$) littermates, as determined by Student's *t* test assuming unequal variances. There was not a significant difference in the number of apoptotic cells between wild-type and $Mtb^{+/-}$ blastocysts. (B and C) Differential interference contrast (B) and fluorescein isothiocyanate (C) images of a single $Mtb^{-/-}$ blastocyst, collapsed because of manipulation.

***Mtb* is required for survival and proliferation of the ICM.** We suspected that the $Mtb^{-/-}$ ICM was undergoing cell death by some mechanism, because we observed a high frequency of cells with pyknotic nuclei in sections of these embryos at E5.5 (Fig. 5 and data not shown). Therefore, $Mtb^{-/-}$ blastocysts were fixed at E3.5, subjected to TUNEL analysis to detect cells undergoing apoptosis, and subsequently genotyped by PCR. $Mtb^{-/-}$ blastocysts did contain a significantly higher number of TUNEL positive nuclei than their wild-type or $Mtb^{+/-}$ littermates (Fig. 6).

If MTB is critical for mediating a cell survival signal in embryonic stem cells, then the absence of p53, a major regulator of cell survival that induces apoptosis, might allow $Mtb^{-/-}$ embryos to survive beyond implantation. The absence of p53 can prolong survival of embryos lacking tumor susceptibility gene *tsg101*, a putative transcriptional corepressor, for 2 days (22). To determine whether loss of p53 could partially rescue the phenotype in mice with a targeted disruption in *Mtb*, we crossed the *Mtb* mutation into a p53 null background and interbred $Mtb^{+/-}$, $p53^{-/-}$ progeny. Embryos from these

crosses were dissected and genotyped by PCR at E6.5. No $Mtb^{-/-}$ embryos were identified from 31 embryos, indicating that the loss of p53 did not rescue the growth defect in $Mtb^{-/-}$ embryos. These data demonstrate that *Mtb* null embryos do not die as a result of a p53-dependent survival defect and support the conclusion that MTB is required for maintenance of the ICM-derived stem cell population in postimplantation development.

Summary. Disruption of the *Mtb* locus results in peri-implantation lethality due to a failure of $Mtb^{-/-}$ embryos to undergo the proliferative burst that generates an egg cylinder upon implantation. Our in vitro analysis of blastocysts isolated from *Mtb* heterozygous crosses demonstrates that the $Mtb^{-/-}$ ICM has a severe growth defect. This growth defect was not rescued by the loss of p53, indicating that $Mtb^{-/-}$ cells exhibit a p53-independent survival defect and/or a proliferation defect. The requirement for MTB in pluripotent cells is further supported by our inability to generate $Mtb^{-/-}$ embryonic stem cell lines. Thus, we have identified a novel gene that plays a key role in early embryonic development in the mouse. The human homologue of MTB, hypothetical protein FLJ20311, is moderately expressed in human embryonic stem cells as well (24a), suggesting that MTB is likely to have a conserved function in mammalian embryo-derived stem cells. Given the expression pattern and phenotype, it is possible that MTB specifically functions in rapidly proliferating tissues, such as the pluripotent cells in early embryos and sites of neurogenesis and hematopoiesis later in embryogenesis and in the adult. The early embryonic lethality of $Mtb^{-/-}$ embryos precludes analysis of MTB in neuronal and hematopoietic tissues. Future studies will address the specific functions of MTB, on a molecular level, in these different cell populations, with particular emphasis on the role of MTB in early embryogenesis.

ACKNOWLEDGMENTS

We thank Steven Droho and Josh Wechsler for excellent technical assistance; Barbara Kee, Harinder Singh, and Brian Kennedy for helpful insights and discussions; and Sandeep Gurbuxani and Steven Kosak for critical reading of the manuscript.

This research was funded, in part, by NIH grant DK61464-01 (to J.D.C) and by a grant from the V Foundation for Cancer Research (to J.D.C.). J.D.C. is a recipient of a Burroughs Wellcome Fund Career Award in the Biomedical Sciences. S.H.O. is an investigator of the Howard Hughes Medical Institute.

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