

Identification and Chromosomal Localization of *Atm*, the Mouse Homolog of the Ataxia–Telangiectasia Gene

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Atm, the mouse homolog of the human ATM gene defective in ataxia–telangiectasia (A–T), has been identified. The entire coding sequence of the *Atm* transcript was cloned and found to contain an open reading frame encoding a protein of 3066 amino acids with 84% overall identity and 91% similarity to the human ATM protein. Variable levels of expression of *Atm* were observed in different tissues. Fluorescence *in situ* hybridization and linkage analysis located the *Atm* gene on mouse chromosome 9, band 9C, in a region homologous to the ATM region on human chromosome 11q22–q23. © 1996 Academic Press, Inc.

INTRODUCTION

Ataxia–telangiectasia (A–T) is a human autosomal recessive disorder affecting multiple systems of the human body. The main features of A–T are cerebellar degeneration, immunodeficiency, acute cancer predisposition, chromosomal instability, radiation sensitivity, and defects in cell cycle checkpoints activated by radiation damage (see Harnden, 1994; Shiloh, 1995, for recent reviews). The A–T locus was mapped to chromosome 11q22–q23 (Gatti *et al.*, 1988), and the responsible gene, ATM, was subsequently identified in our laboratory by positional cloning (Savitsky *et al.*, 1995a,b). ATM extends over 150 kb and produces a major transcript of about 13 kb (Uziel *et al.*, 1995; Savitsky *et al.*, 1995a,b, and unpublished data). This transcript contains an open reading frame of 9168 nucleotides predicting a large protein containing 3056 amino acids, with a molecular mass of 350 kDa and a carboxy-terminus region showing similarity to the catalytic domain

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of the signal transduction mediator phosphatidylinositol 3-kinase (PI 3-kinase). Such a domain is also observed in several large proteins in yeast, *Drosophila*, and mammals that are involved in maintenance of genome stability, cellular responses to DNA damage, or cell cycle regulation (Savitsky *et al.*, 1995b; Zakian, 1995).

No clinical entity corresponding to A–T has been described to date in the mouse. We have identified the murine ATM homolog, *Atm*, and report here the sequence of its coding region and its chromosomal location.

MATERIALS AND METHODS

Library screening. An oligo(dT)-primed mouse brain cDNA library in a Uni-Zap XR vector, a mouse 129Sv genomic library (Stratagene, San Diego, CA), and a randomly primed mouse brain cDNA library in λ gt10 (Clontech, Palo Alto, CA) were used; 10^6 PFU were screened with each probe. The libraries were plated at a density of 5×10^4 PFU per 140-mm plate, and two sets of replica filters were made using Qiabran nylon membranes (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Filters were prehybridized for 2 h at 65°C in $6\times$ SSC, $5\times$ Denhardt's, 1% *N*-laurylsarcosyl, 10% dextran sulfate, and 100 μ g/ml sheared salmon sperm DNA. Hybridization was performed at 65°C for 16–18 h in the same solution containing 10^6 cpm/ml of probe labeled with [³²P]dCTP by random priming. Final washes were performed for 30 min in $0.5\times$ SSC, 0.1% SDS at 50°C. Positive clones were plaque-purified using standard techniques.

RT-PCR. First-strand synthesis was performed using 2 μ g of total RNA from mouse 3T3 cells with an oligo(dT) primer and Superscript II (Gibco-BRL, Gaithersburg, MD). The reaction products served as templates for PCR with gene-specific primers.

Sequence analysis. The insert of cDNA clone 15-1 (see below) was excised from a gel, self-ligated to form concatemers, and sonicated to obtain random fragments. These fragments were size-fractionated by gel electrophoresis, and the 1.0- to 1.5-kb fraction was extracted from the gel and subcloned in a pBluescript vector (Stratagene). The end portions of individual clones were sequenced with vector-specific primers in an automated sequencer (Model 373A, Applied Biosystems Division, Perkin–Elmer), and the sequences were aligned with the AutoAssembler program (Applied

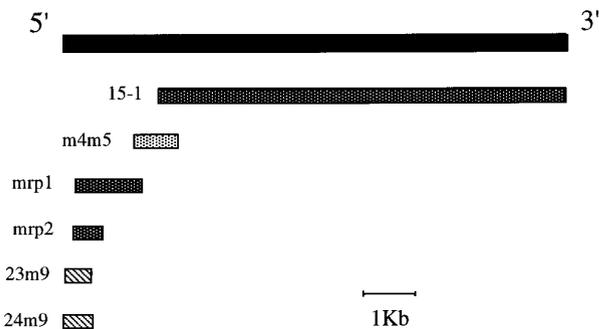


FIG. 1. Molecular cloning of the coding region of the *Atm* transcript. The solid box depicts the entire length of the cloned sequence. (▨) cDNA clones. (▩) RT-PCR product. (▧) PCR products obtained from a cDNA library. See text for details.

Biosystems). In the final sequence, each nucleotide position represents at least three independent overlapping readings. In smaller cDNA inserts, sequencing was initiated with vector-specific primers, and additional sequencing primers were designed for both strands as sequencing progressed. Sequencing of RT-PCR products was performed with the PCR primers.

Fluorescence in situ hybridization (FISH). Preliminary chromosomal localization of the *Atm* gene was determined by FISH analysis. Mouse metaphase chromosomes were prepared from concanavalin A-stimulated lymphocytes obtained after splenectomy as described (Boyle *et al.*, 1992), with slight modifications. Briefly, homogenized spleen tissue was cultured for 48 h in RPMI 1640 medium supplemented with 20% fetal bovine serum, 6 μ g/ml concanavalin A, and 86.4 μ M β -mercaptoethanol. The cell cycle was synchronized by incubation with methotrexate (17 h, 4.5 mM). The S-phase block was released with BrdU (30 μ M) and FUDR (0.15 μ g/ml) for 5 h. Colcemid was added for 10 min; the cells were incubated in KCl (0.55%) and fixed with methanol:acetic acid (3:1).

The mouse *Atm* genomic clone used for FISH analysis was obtained by screening the mouse 129Sv genomic library with a human 236-bp PCR probe corresponding to nt 5381–5617 of the human *ATM* cDNA. Sequence analysis confirmed that this clone contains a 177-bp exon corresponding to nt 5705–5881 of the mouse *Atm* cDNA.

The mouse *Atm* genomic clone was labeled by nick-translation with digoxigenin-11-dUTP (Boehringer Mannheim, Indianapolis, IN). To facilitate chromosome identification, a biotinylated mouse chromosome 9-specific painting probe (Vector Laboratories, Burlingame, CA) was used for cohybridization. The probe sequences and metaphase chromosomes were heat denatured separately. Hybridization was performed for 15 h at 37°C in a solution containing 50% formamide, 2 \times SSC, and 10% dextran sulfate. Posthybridization washes were performed as described (Ried *et al.*, 1992). The biotinylated probe sequences were detected by incubation with avidin conjugated to FITC (Vector Laboratories), and the digoxigenin-labeled sequences were detected by incubation with mouse anti-digoxin and goat anti-mouse conjugated to TRITC (Sigma Chemicals, St. Louis, MO). Chromosomes were counterstained with DAPI. The fluorescent signals were sequentially acquired using a cooled CCD camera (Photometrics, Tucson, AZ) coupled to a Leica DMRBE microscope. Gray scale images were converted to tintscale using Gene Join (Ried *et al.*, 1992).

Linkage analysis. Interspecific backcross progeny were generated by mating (C57BL/6J \times *Mus spretus*) F₁ females and C57BL/6J males, as described (Copeland and Jenkins, 1991). A total of 205 N₂ mice were used to map the *Atm* locus. Southern blot analysis was performed as described (Jenkins *et al.*, 1982). All blots were prepared with Hybond-N⁺ membrane (Amersham). The *Atm* probe, REF3, a PCR-amplified fragment from the *Atm* mouse cDNA representing nt 6000–7264, was labeled with [α -³²P]dCTP using a random priming labeling kit (Stratagene); washing was performed to a final stringency of 0.5 \times SSCP, 0.1% SDS, 65°C. Fragments of 4.9, 3.6, and 1.4 kb were detected in *Hind*III-digested C57BL/6J DNA, and fragments of 5.6 and 4.3 kb were detected in *Hind*III-digested *M. spretus* DNA.

The presence or absence of the 5.6- and 4.3-kb *M. spretus*-specific fragments, which cosegregated, was followed in the backcross mice.

A description of the probes and RFLPs for the loci linked to *Atm*, including glutamate receptor, ionotropic kainate 4 (Grik4); thymus cell antigen-1 θ (Thy1); Casitas B-lineage lymphoma (Cbl); CD3 antigen, γ polypeptide (Cd3g); and dopamine receptor 2 (Drd2), has been reported previously (Kingsley *et al.*, 1989; Regnier *et al.*, 1989; Szpirer *et al.*, 1994). The mouse chromosomal locations of mitochondrial acetoacetyl-CoA thiolase (Acat1) and src-kinase (Csk) were determined for the first time and are reported in this paper. Recombination distances were calculated as described (Green, 1981), using the computer program SPRETUS MADNESS. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

The Csk probe, a 2.2-kb *Eco*RI/*Xho*I fragment derived from the mouse cDNA (Thomas *et al.*, 1991), was labeled with [α -³²P]dCTP using a nick-translation labeling kit (Boehringer Mannheim); washing was performed to a final stringency of 0.1 \times SSPE, 0.1% SDS, 65°C. A fragment of 9.4 kb was detected in *Hind*III-digested C57BL/

1	MSLALNDLLI	CCRQLEHEDRA	TERRKEVDKF	KRLIQDPETV	QHLDRHSDSK
51	QGKYLNDWAV	FRFLQKYIQK	EMESLRTAKS	NVSATQSSR	QKKMQEISSL
101	VRYF1KCANR	RAPRLKQDLD	LNVMVDTVKD	SSNGLTGADQ	CSNILLKDLI
151	SVRKVWCEVS	QQQWLELFSL	YFRLYLKPQD	DINRVLVARI	IHAVTRGCCS
201	QTDLGPKSFL	DLFSKAIQYA	RQEKSSPGLS	HLLAALNIFL	KSLAVNFRKR
251	VCEAGDEILP	TLLYIWTQHR	LNDSLKVEII	ELIQLQIYIH	HPQGARAPEE
301	GAYESMKWKS	ILYNLYDLLV	NEISHGSRG	KYSSGSRNIA	VKENLIDLMA
351	DICYQLFDAD	TRSVIEISQY	VTQRESTDYS	VPCKRRKIDV	GWEVLIKDYLO
401	KSQSDFDLVP	WLQITTRLIS	KYRSSLNPNCE	LSPLILILYQ	LLPQRRGER
451	IPYVLRCLKE	VALCQGGKSN	LESSQKSDLL	KLWIKIWSIT	FRGISSGGTQ
501	TENFGLLEAI	TQSSLVLELDR	EFWKLFTGSA	CKPSSPSVCC	LTLALSICVV
551	GAYEADPSFK	SVCEANRFSF	VKESIMRWLL	FYQLEDDLED	STELPPLLQR
601	NFPHLVVEKI	LVSLTMKNKX	AAMKFFQSPV	ECEQHCEDEK	EPFSEVEVEL
651	FLQTTDFDKM	FLPTVKEYAV	EKFPSSVGFPS	VQONLKESSL	HYLLGLSEQL
701	LSNYSSEITS	SETLVRCSSL	LVGVLCGYCY	MGIITEDEAH	KSELFQKAKS
751	LMQACAGESI	LRKNKTNEES	RIGSLRNVMH	LCTSCLCTHE	KHTPNLISAG
801	FFLLRLTSEK	MNDIADICKS	LASCTKRPDL	HGVHPGEDDE	DGGGCDLSME
851	AEGPSSSTGLS	TAYPASSVSD	ANDYGENQNA	VGAMSPILAD	YLSKQDHLL
901	DMRLFLGRSV	TASQSHTVSF	RGADIRRKLK	LLDSSDILD	MKPLLHMHYL
951	VLLKDLPGNE	HSLPMEDVVE	LLQPLSLVCS	LHRRDQDVCK	TILSNVLHIV
1001	TNLGQGSVDM	ESTRIAQGHF	LTVMGAFWHL	TKERKCVFSS	RAGLVKCLQT
1051	LLEADPTSEW	AILNVKGFDF	PVNEAFSQFL	ADDDHQQVRL	AASVNRLLFQ
1101	DMRGDGFSSR	LKALPLKQFQ	TSFNNAYTFA	EAGIRGLLCD	SQNPDLLEDE
1151	YNRKSVLLMM	IAVVLHCSPV	CEKQALFALC	KSVKENRLEP	HLVKKVLEKVI
1201	SESFGCRSLE	DFMISHLDYL	<u>VLEWLNLDQT</u>	<u>EYSLSSFFPM</u>	<u>LLN</u> YTSIEDF
1251	YRSCYKILIP	HLVIRSHFDE	VKSTANQIQK	CWKSLLDVCF	PNLVLHLLPV
1301	FAYEGTRDSY	VSQKRETATK	VYDITLKGEDF	LGKQIDQVFI	SNLPEIVVEL
1351	LMTLHETADS	ADSDASQAT	ALCDFSGDL	PAPNPPYFVS	HVIQATFAYI
1401	SNOCHTKFKS	ILEILSKIPD	SYQKILLATA	EQAAETNVPF	KKHRKILYIH
1451	LFVSLLLKDI	QSGLGGAWAF	VLRDVIYTLI	HYINKRSSHF	TDVSLRSFSL
1501	CCDLSRVCH	TAVTQCKDAL	ESHLLHIVGT	LIPLVLDYQE	QEQVLDLLKY
1551	LVIDNKNKNN	LSVTIKLLDP	FPDHIYFKDL	RLTQQKIKYS	GGPFSLLLEEI
1601	NHFLSVSAYN	PLPLTRLGLE	KDLRRLEQHQ	KDQMLDLRFA	SQNPQDQDV
1651	VKLUVSLMLQ	SKMAVNQTEG	REVLVAEGRC	LGEITGLDFS	TIAVGHKIDV
1701	SYTKAYGLPE	DRELQWTLIM	LTALNNTLVE	DSVKIRSAAA	TCLKNILATK
1751	IGHIFWENYK	TSADPMLTYL	QPFRTSRKFF	LEVPRSVKED	VLEGLDAMLV
1801	WPQFSHSDI	WIKTLTCAFL	DSGGINKNSL	QLLKPMPCEVK	TDFCQVLLPY
1851	LHIDVLLQDT	HESWRTLASA	HVRRFTTSCF	KHSSQAQRSA	TPANSDESSE
1901	NFLRCCLDKK	SQRTMLAVDV	YLRRQKRPSS	GFADDFAWL	DLNLYEVAKV
1951	AQCSAHTFA	LLYAEIYSDK	KSTDEQEKRS	PTFEEGSGQT	TISLSEKSK
2001	EETGISLQDL	LLEIYRSIGE	PDSLQYCGGG	KMLQPLTRLR	DYEHREATWEK
2051	ALVTYDLETS	ISSSTRSQSI	IQALNKLKSL	HILSVYLKGL	TRIEEREWCAE
2101	LQELRYQAAV	RNMQWGLCAS	AGQEVGYTSP	HESLYNALQC	LRNREFSTFY
2151	ESLRYASLFR	VKEVEELSGS	ALQEVYSLYP	TLRSLQAIGE	LRNSGBLFSR
2201	SVPDRERSEA	YWKWQKHSQ	LKDSDFSPQE	PLMALRTVIL	ETLVQKEMER
2251	SQGACSKDIL	TKHLVEFVSL	ARTFKNTQLP	ERATFKIKQP	NSAICGISIEW
2301	HLEEAQFWFA	KREQSLALS	LKQMTIKNSL	SPKKNENDAG	LKVIYAECLR
2351	VCGSWLAETC	LENPAVIMQT	YLEKAVKVG	SYDGNSSRELH	NGQMKAFLSL
2401	AFRSDTQYQR	IENYMKSEF	ENKQTLKRA	KEVGLLREH	KIQTNRITYTK
2451	VQRELELDEC	ALRALREDRK	RFLCKAVENY	INCLLSGEEH	DLWVFRCLSL
2501	WLENSGVSEV	NGMMKKDGMK	ISSYKFLPLM	YQLAARMGTX	MTGGGLGFHEV
2551	LNNLISRISL	DHPHTLFFII	LALANANDE	FLSKPNETTR	SRITKTSKSE
2601	NSHLDEDRTE	AATRIIHSNR	SKRCMKVMD	EALCDAYIIL	ANMDASQWRA
2651	QRGINDINPR	QPITIKLNLE	DVVVPTMIEK	TDPTGAYENL	VIKSFTEFEF
2701	RLAGGLNLPK	IIDCVGSDGK	ERRQLVGRD	DLRQDAVMQ	VFQMCNTLLQ
2751	RNFETRKRKL	TICTYKVVPL	QRSQGVLEWC	GTVPVIGEYL	VNSDEGAHRR
2801	YRPNDFSANQ	CQKKMMEVQK	KSFBEKYDTF	MTICQNFEPV	FRYFCMEKFL
2851	DVAFVEQEKRL	AYTRSVATSS	<u>IVGYLILGMI</u>	<u>RVQNI</u> ILINE	QSAELVHIDL
2901	GPAAEFGKLL	PIPTVPFRLS	SRDVIYDGMG	THGVGFVRRK	CSBKTEVMVRS
2951	SQETLLTIVE	VLLYDPLFDW	TMNPLKALYL	QQRPEBESDL	HSTPNADDQE
3001	CKQSLSDTDF	SFNKVAERVL	MRLQEKLGKV	EEGTVLSVGG	QVNLLIQQAM
3051	DPKNLSRFLP	GWKAAVV			

FIG. 2. Amino acid sequence of the *Atm* protein. The leucine zipper and the PI 3-kinase signature are underlined and shown in boldface letters.

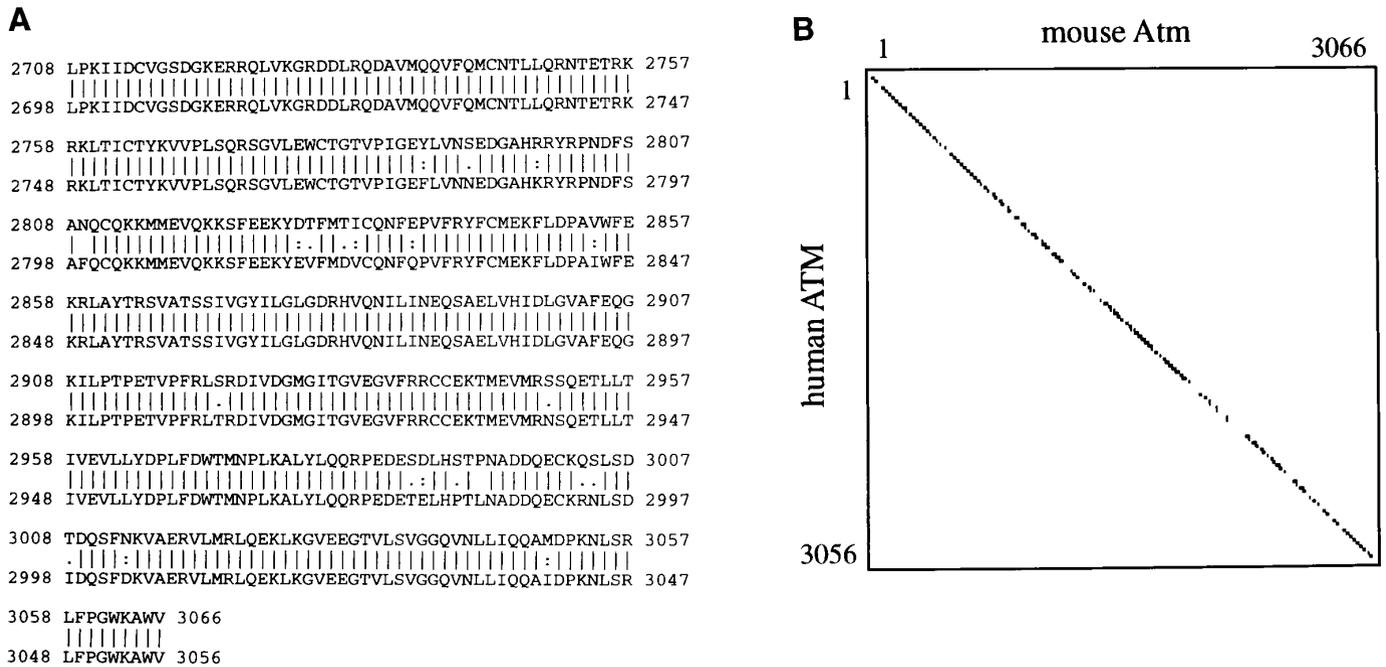


FIG. 3. Comparison of amino acid sequences of the human ATM and mouse *Atm* proteins. (A) Alignment of amino acid sequences spanning the carboxy-terminal portions that contain the PI 3-kinase domains of the two proteins. Identical amino acids are highlighted by vertical bars, and similar amino acids by one or two dots. (B) A dot plot showing an amino acid sequence comparison throughout the entire length of both proteins, obtained using the PROSIS program. Sequential blocks of 12 amino acids were compared, and a dot was marked on the diagonal when 11 of 12 residues were identical in both sequences.

6J DNA, and a fragment of 5.8 kb was detected in *Hind*III-digested *M. spretus* DNA. The presence or absence of the 5.8-kb *M. spretus*-specific fragment was followed in the backcross mice. The *Acat1* probe, a 1.4-kb fragment from the *Acat* rat cDNA (Fukao *et al.*, 1990), was labeled by nick-translation and washed from the blots to a final stringency of $0.8\times$ SSCP, 0.1% SDS, 65°C. A fragment of 23 kb was detected in *Eco*RI-digested C57BL/6J DNA, and fragments of 22 and 5.4 kb were detected in *Eco*RI-digested *M. spretus* DNA. The presence or absence of the 22- and 5.4-kb *M. spretus*-specific fragments, which cosegregated, was followed in the backcross mice.

RESULTS

Molecular Cloning of the Coding Sequence of *Atm* Gene

In search of a cDNA clone derived from a murine gene corresponding to the human ATM, 10^6 PFU from

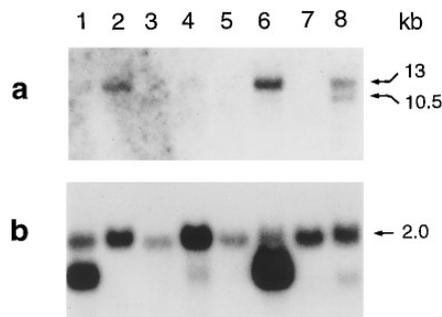


FIG. 4. Expression of the *Atm* gene. (a) A Northern blot representing various mouse tissues was probed with a fragment spanning nucleotides 2297–5311 of the *Atm* transcript. (b) The same blot was probed with an actin probe. The upper band of 2.0 kb was used to assess the differences in RNA amounts between lanes. 1, heart; 2, brain; 3, spleen; 4, lung; 5, liver; 6, skeletal muscle; 7, kidney; 8, testis.

a mouse brain cDNA library were screened with a PCR product corresponding to nt 4021–8043 of the human ATM cDNA (Savitsky *et al.*, 1995b); the first nucleotide of the open reading frame was numbered 1). Fifteen positive clones were identified, and the longest one, of 8.5 kb (designated 15-1; Fig. 1), was further analyzed. High-stringency hybridization of this clone to panels of radiation hybrids, YAC, and cosmid clones representing the human ATM locus (Rotman *et al.*, 1994; Shiloh, 1994; Savitsky *et al.*, 1995a,b) (not shown) showed strongly hybridizing sequences within the ATM locus. Northern blotting analysis and subsequent sequencing and alignment with the human ATM transcript (see below) confirmed that 15-1 corresponded throughout its length to the human gene but was missing the 5' end of the corresponding mouse transcript.

Screening of a randomly primed mouse brain cDNA library with a probe corresponding to the 5' region of the human ATM transcript (nt 1–2456) identified two clones, MRP1 and MRP2, of 1.3 and 0.6 kb, respectively (Fig. 1). The gap between clones 15-1 and MRP1 was subsequently bridged using RT-PCR with primers derived from these clones, which produced the fragment m4m5 of 840 bp. Finally, a primer derived from the MRP1 sequence was designed and used with vector-specific primers to obtain two PCR products, 23m9 and 24m9, from the randomly primed brain cDNA library. All these clones and PCR products hybridized exclusively to the ATM locus in the human genome (not shown). Their sequences were assembled and formed a contig of 9620 nucleotides (Fig. 1; GenBank Accession No. U43678).

Sequence Comparisons

The sequence of the contig shown in Fig. 1 shows an open reading frame (ORF) of 9201 nt and includes a 41-nt 5' UTR and a 378-nt 3' UTR. These UTRs are probably not complete, in view of the length of the UTRs of the ATM transcript and the lack of a poly(A) tail in 15-1. The ORF encodes a putative protein of 3066 amino acids with a molecular mass of 349.5 kDa (Fig. 2). When the nucleotide and amino acid sequences corresponding to the coding regions of the mouse and human ATM transcripts were aligned, there was an overall identity of 85% at the nucleotide sequence level and 84% identity and 91% similarity at the amino acid level. The difference of 10 amino acids between the human and the mouse proteins is the net sum of several insertions and deletions in both proteins when compared to each other. The PI 3-kinase domain found in ATM and other related proteins was identified in the mouse sequence, as was the leucine zipper present in the human ATM protein (Fig. 2). These results indicated that we had obtained the entire coding sequence of *Atm*, the murine homolog of the human ATM gene. It is noteworthy that the human and mouse proteins were most similar within the PI 3-kinase domain at the carboxy terminus (94% identity, 97% similarity), while the other portions of these proteins showed variable identity and similarity, reaching a minimum of 70 and 82%, respectively, in some regions (Fig. 3).

Expression Pattern

A Northern blot representing several mouse tissues (Clontech) was probed with a fragment representing nt 2297–5311 of the *Atm* transcript (Fig. 4). This probe identified a message of about 13 kb in brain, skeletal muscle, and testis that was barely detectable in heart, spleen, lung, and kidney. In the testis, another band of about 10.5 kb was observed at about 50% intensity compared to that of the 13-kb band (Fig. 4). This pattern seems to represent greater differences in expression levels between tissues compared to the more uniform pattern observed in human tissues (Savitsky *et al.*, 1995a). In addition, the 10.5-kb band, which may represent mRNA species with alternative polyadenylation (Savitsky *et al.*, in preparation), was not detected in any of 16 human tissues tested previously, but was clearly observed in cultured human fibroblasts (Savitsky *et al.*, 1995a).

Chromosomal Localization of the *Atm* Gene by FISH

Initial chromosomal localization of the mouse *Atm* gene was determined by dual-color FISH. A digoxigenin-labeled probe was cohybridized with a chromosome painting probe specific for mouse chromosome 9, which confirms the identification of DAPI-stained mouse chromosomes. Mouse chromosome 9 contains homologous regions of human chromosomes 11q, including 11q22–q23, the region to which the human ATM gene was assigned. Twelve randomly selected

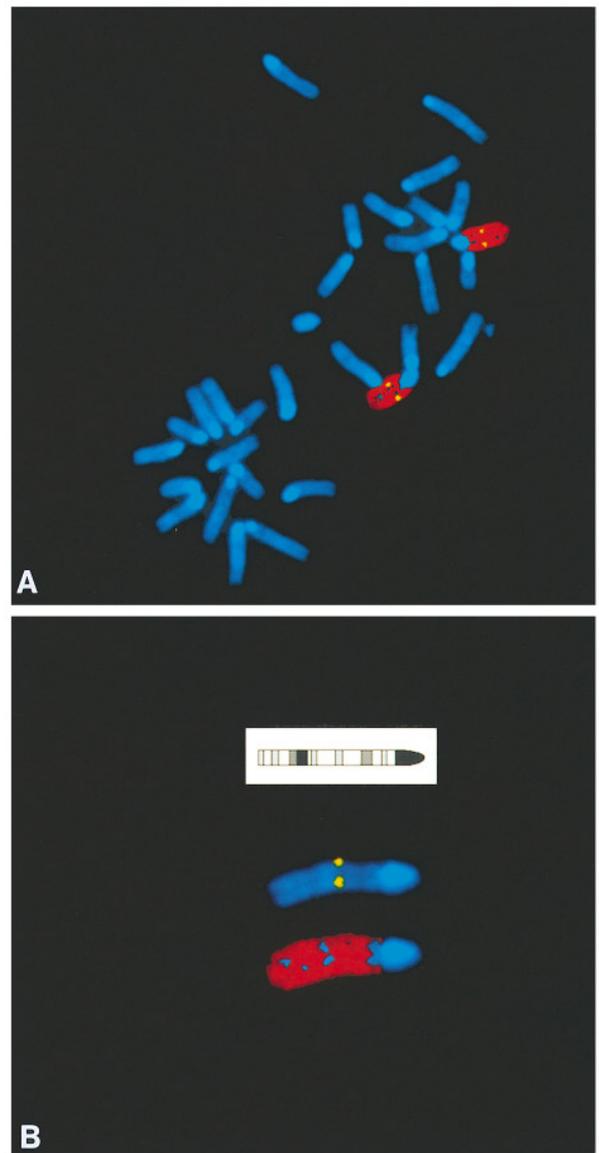


FIG. 5. (A) Mouse metaphase spread after dual-color fluorescence *in situ* hybridization using the mouse *Atm* probe (pseudocolored in yellow) and a mouse chromosome 9-specific painting probe (pseudocolored in red). (B) Separate visualization of the hybridization on banded mouse chromosomes. The signal localizes to chromosomal band 9C. The idiogram depicts chromosome 9 schematically.

metaphases were analyzed. Signals were observed in 90% of the cells on mouse chromosome 9C (Fig. 5). Other chromosomal positions were not observed.

Genetic Mapping of the *Atm* Gene

The *Atm* gene was further localized on the genetic map of mouse chromosome 9 using interspecific backcross analysis and progeny derived from matings of [(C57BL/6J × *M. spretus*) F₁ × C57BL/6J] mice. This interspecific backcross mapping panel has been typed for over 2000 loci that are well distributed among all the autosomes as well as the X chromosome (Copeland and Jenkins, 1991). C57BL/6J and *M. spretus* DNAs were digested with several enzymes and analyzed by

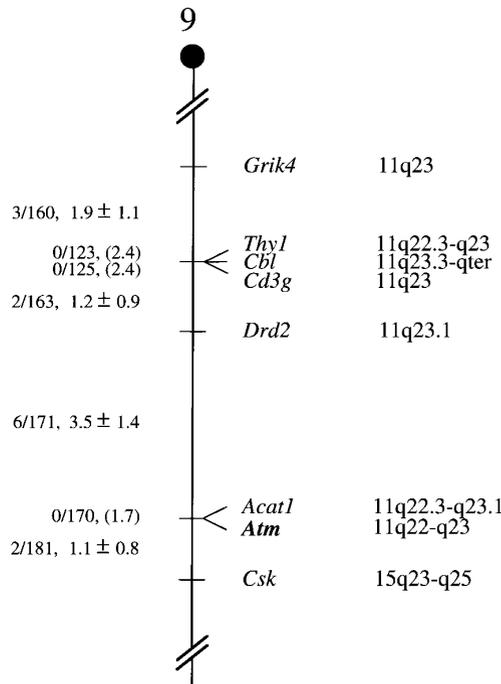
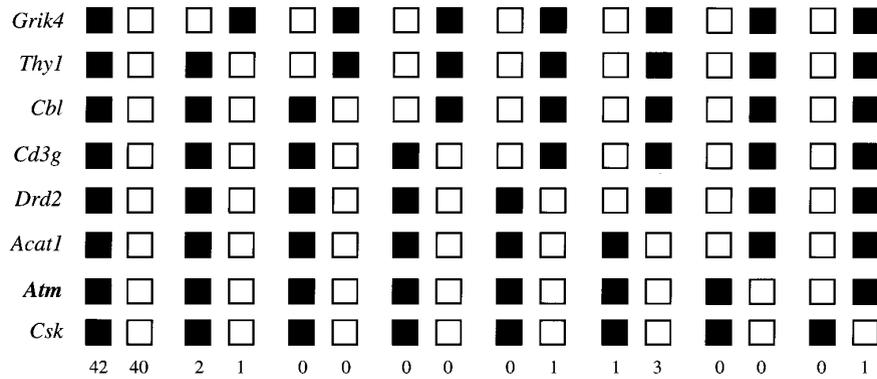


FIG. 6. Chromosomal location of *Atm* in the mouse genome. The locus was mapped by interspecific backcross analysis. The segregation patterns of *Atm* and flanking genes are shown at the top. Each column represents the chromosome identified in the backcross progeny that was inherited from the (C57BL/6J × *M. spretus*) F₁ parent. The black boxes represent the presence of a C57BL/6J allele, and the white boxes represent the presence of a *M. spretus* allele. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. A partial chromosome 9 linkage map showing the location of *Atm* in relation to linked genes is shown at the bottom. The number of recombinant N₂ animals over the total number of N₂ animals typed plus the recombination frequencies, expressed as genetic distance in centimorgans (± one standard error), is shown for each pair of loci to the left of the map. When no recombinants were found between loci, the upper 95% confidence limit of the recombination distance is given in parentheses. The positions of loci in human chromosomes are shown to the right of the map. References for the human map positions of loci cited in this study can be obtained from GDB (Genome Database), a computerized database of human linkage information maintained by The William H. Welch Medical Library of Johns Hopkins University (Baltimore, MD).

Southern blot hybridization for informative restriction fragment length polymorphisms (RFLPs), using a probe representing nt 6000–7264 of the *Atm* transcript.

The results indicated that *Atm* is located in the proximal region of mouse chromosome 9 linked to *Grik4*, *Thy1*, *Cbl*, *C3g*, *Drd2*, *Acat1*, and *Csk* (Fig. 6). Ninety-one mice were analyzed for every marker and are shown in the segregation analysis (Fig. 6); however, up to 203 mice were typed for some pairs of markers. Each locus was analyzed in pairwise combinations for recom-

bination frequencies using the additional data. The ratios of the total number of mice analyzed for each pair of loci and the recombination frequencies between the loci are shown in Fig. 6.

DISCUSSION

The ATM gene is highly conserved across a wide range of species, as indicated by hybridization analysis (Y. Ziv *et al.*, unpublished data). A growing family of yeast and *Drosophila* proteins that typically contain

the PI 3-kinase domain was recently found to be involved in cellular phenotypes that partly overlap that of A-T (Savitsky *et al.*, 1995b; Zakian, 1995). It is not clear, however, if these proteins are the true ATM homologs in these organisms, since the sequence similarities between them and ATM are less pronounced outside the PI 3-kinase domain. The degree of sequence similarity throughout the entire length of the mouse protein reported here and the human ATM protein, as well as the chromosomal location of the mouse gene, indicates that this gene is indeed the mouse homolog of the human gene responsible for A-T.

Two mapping methods were used to assign the *Atm* gene to chromosome 9, band 9C. Comparative gene mapping in mouse and human has revealed numerous regions of homology between the two species (Copeland *et al.*, 1993). This is clearly demonstrated between this portion of mouse chromosome 9 and human chromosome 11q22-q23. The human homologs of *Grik4*, *Thy1*, *Cbl*, *Cd3g*, *Drd2*, *Acat1*, and *Atm* map to 11q22-q23. It is noteworthy that, similar to the close map locations of *Atm* and *Acat1* in the mouse, ATM and ACAT1 lie about 200 kb apart in the human genome (A. Bar-Shira *et al.*, unpublished data). The mapping of *Atm* refines the distal end of the human 11q22-q23 homology unit. *Csk*, 1.1 cM distal to *Acat* and *Atm*, maps to human chromosome 15q23-q25. The average length of a conserved autosomal segment in mice was estimated at 8.1 cM (Nadeau and Taylor, 1984). The conserved segment on mouse chromosome 9, which corresponds to 11q22-q23 in humans, extends centromeric to *Grik4* and spans approximately 19 cM.

The high degree of conservation between the human and the mouse proteins suggests similar roles; however, the difference in expression patterns between mice and humans suggested by our Northern results may lead to differences between the phenotypes associated with these proteins in the two organisms. To date, no phenotype identical to A-T has been reported in the mouse. We compared our chromosome 9 interspecific map with a composite mouse linkage map from the Mouse Genome Database (The Jackson Laboratory, Bar Harbor, ME), which reports the location of many uncloned mouse mutations. Only one uncloned mouse mutation, *luxoid* (*lu*), lies in the vicinity of *Atm*, but this skeletal abnormality is highly unlikely to represent a mouse disorder corresponding to A-T. The mouse phenotype closest to A-T is severe combined immune deficiency on mouse chromosome 16. It is characterized by a deficiency in mature B and T lymphocytes, radiation sensitivity, chromosomal instability, defective rejoining of DNA double-strand breaks, and defective V(D)J recombination (Bosma and Carroll, 1991). This phenotype is indeed caused by defects in one of the phenotypes with a PI 3-kinase domain, the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) (Blunt *et al.*, 1995; Hartley *et al.*, 1995). The reason for the lack of a mouse phenotype associated with the *Atm* gene may be that, unlike in humans, such a phenotype is either embryonic lethal or considerably milder than

that in humans. Attempts currently in progress to construct a mouse model of A-T by targeted inactivation of *Atm* may provide an answer to this question.

Identification of the *Atm* gene should lead to better understanding of its evolutionary aspects and developmental regulation. It will also enable manipulation of this gene in the mouse, which should provide better understanding of the molecular pathology of A-T.

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REFERENCES

- Ashley, C. T., Sutcliffe, J. S., Kunst, C. B., Leiner, H. A., Eichler, E. E., Nelson, D. L., and Warren, S. T. (1993). Human and murine FMR-1: Alternative splicing and translational initiation downstream of the CGG-repeat. *Nature Genet.* 4: 244-251.
- Bennett, L. M., Haugen-Strano, A., Cochran, C., Brownlee, H., Fiedorek, F. T., Jr., and Wiseman, R. W. (1995). Isolation of the mouse homologue of BRCA1 and genetic mapping to mouse chromosome 11. *Genomics* 29: 576-581.
- Blunt, T., Finnie, N., Taccioli, G. E., Smith, G. C., Demengeot, J., Gottlieb, T. M., Mizuta, R., Varghese, A. J., Alt, F. W., Jeggo, P. A., and Jackson, S. P. (1995). Defective DNA-dependent protein kinase activity is linked to V(D)J recombination and DNA repair defects associated with the murine scid mutation. *Cell* 80: 813-823.
- Bosma, M. J., and Carroll, A. M. (1991). The SCID mouse mutant: Definition, characterization, and potential uses. *Rev. Immunol.* 9: 323-350.
- Boyle, A., Feltquite, D. M., Dracopoli, N. C., Housman, D. E., and Ward, D. C. (1992). Rapid physical mapping of cloned DNA on banded mouse chromosomes by fluorescence *in situ* hybridization. *Genomics* 12: 106-115.
- Copeland, N., and Jenkins, N. (1991). Development and applications of a molecular genetic linkage map of the mouse genome. *Trends Genet.* 7: 113-118.
- Copeland, N. G., Jenkins, N. A., Gilbert, D. J., Eppig, J. T., Maltais, L. J., Miller, J. C., Dietrich, W. F., Weaver, A., Lincoln, S. E., Steen, R. G., Stein, L. D., Nadeau, J. H., and Lander, E. S. (1993). A genetic linkage map of the mouse: Current applications and future prospects. *Science* 262: 57-66.
- Derry, J. M. J., Wiedemann, P., Blair, P., Wang, Y., Kerns, J. A., Lemahieu, V., Godfrey, V. L., Wilkinson, J. E., and Francke, U. (1995). The mouse homolog of the Wiscott-Aldrich syndrome protein (WASP) gene is highly conserved and maps near the scurfy (*sf*) mutation on the X chromosome. *Genomics* 29: 471-477.
- Fukao, T., Yamaguchi, S., Kano, M., Orii, T., Fujiki, Y., Osumi, T., and Hashimoto, T. (1990). Molecular cloning and sequence of the complementary DNA encoding human mitochondrial acetoacetyl-coenzyme A thiolase and study of the variant enzymes in cultured fibroblasts from patients with 3-ketothiolase deficiency. *J. Clin. Invest.* 86: 2086-2092.
- Gatti, R. A., Berkel, I., Boder, E., Braedt, G., Charmley, P., Concan-

- non, P., Foroud, T., Jaspers, N. G. J., Lange, K., Lathrop, G. M., Leppert, M., Nakamura, Y., O'Connell, P., Paterson, M., Salser, W., Sanal, O., Silver, J., Sparkes, R. S., Susi, E., Weeks, D. E., Wei, S., White, R., and Yoder, F. (1988). Localization of an ataxia-telangiectasia gene to chromosome 11q22-23. *Nature* 336: 577-580.
- Green, E. L. (1981). Linkage, recombination and mapping. In "Genetics and Probability in Animal Breeding Experiments," pp. 77-113, Oxford Univ. Press, New York.
- Harnden, D. G. (1994). The nature of ataxia-telangiectasia: Problems and perspectives. *Int. J. Radiat. Biol.* 66: S13-S19.
- Hartley, K. O., Gell, D., Smith, G. C. M., Zhang, H., Divecha, N., Connelly, M. A., Admon, A., Lees-Miller, S. P., Anderson, C. W., and Jackson, S. P. (1995). DNA-dependent protein kinase catalytic subunit: A relative of phosphatidylinositol 3-kinase and the ataxia telangiectasia gene product. *Cell* 82: 849-856.
- Jenkins, N. A., Copeland, N. G., Taylor, B. A., and Lee, B. K. (1982). Organization, distribution and stability of endogenous ecotropic murine leukemia virus DNA sequences in chromosomes of *Mus musculus*. *J. Virol.* 43: 26-36.
- Kingsley, D. M., Jenkins, N. A., and Copeland, N. G. (1989). A molecular genetic linkage map of mouse chromosome 9 with new regional localizations for *Gsta*, *T3g*, *Ets-1*, and *Ldlr* loci. *Genetics* 123: 165-172.
- Laval, S. H., Blair, H. J., Hirst, M. C., Davies, K. E., and Boyd, Y. (1992). Mapping of FMR1, the gene implicated in fragile X-linked mental retardation, on the mouse X chromosome. *Genomics* 12: 818-821.
- Levinson, B., Vulpe, C., Elder, B., Martin, C., Verley, F., Packman, S., and Gitschier, J. (1994). The mottled gene is the mouse homologue of the Menkes disease gene. *Nature Genet.* 6: 369-373.
- Lin, B., Nasir, J., MacDonald, H., Hutchinson, G., Graham, R. K., Rommens, J. M., and Hayden, M. R. (1994). Sequence of the murine Huntington disease gene: Evidence for conservation, and polymorphism in a triplet (CCG) repeat alternate splicing. *Hum. Mol. Genet.* 3: 85-92.
- Mercer, J. F. B., Grimes, A., Ambrosini, L., Lockhart, P., Paynter, J. A., Dierick, H., and Glover, T. W. (1994). Mutations in the murine homologue of the Menkes gene in dappled and blotchy mice. *Nature Genet.* 6: 374-378.
- Nadeau, J. H., and Taylor, B. A. (1984). Lengths of chromosomal segments conserved since divergence of man and mouse. *Proc. Natl. Acad. Sci. USA* 81: 814-818.
- Nasir, J., Lin, B., Bucan, M., Koizumi, T., Nadeau, J. H., and Hayden, M. R. (1994). The murine homologues of the Huntington disease (*Hdh*) and the α -adducin gene (*add1*) map to mouse chromosome 5 within a region of conserved synteny with human chromosome 4p16.3. *Genomics* 22: 198-201.
- Regnier, D. C., Kozak, C. A., Kingsley, D. M., Jenkins, N. A., Copeland, N. G., Langdon, W. Y., and Morse, H. C., III (1989). Identification of two murine loci homologous to the *v-abl* oncogene. *J. Virol.* 63: 3678-3682.
- Ried, T., Baldini, A., Rand, T. C., and Ward, D. C. (1992). Simultaneous visualization of seven different DNA probes using combinatorial labeling and digital imaging microscopy. *Proc. Natl. Acad. Sci. USA* 89: 1388-1392.
- Rotman, G., Savitsky, K., Ziv, Y., Cole, C. G., Higgins, M. J., Bar-Am, I., Dunham, I., Bar-Shira, A., Vanagaite, L., Shinzen, Q., Zhang, J., Nowak, N. J., Chandrasekharappa, S. C., Lehrach, H., Avivi, L., Shows, T. B., Collins, F. S., Bentley, D. R., and Shiloh, Y. (1994). A YAC contig spanning the ataxia-telangiectasia locus (groups A and C) at 11q22-23. *Genomics* 24: 234-242.
- Savitsky, K., Bar-Shira, A., Gilad, S., Rotman, G., Ziv, Y., Vanagaite, L., Tagle, D. A., Smith, S., Uziel, T., Sfez, S., Ashkenazi, M., Pecker, I., Frydman, M., Harnik, R., Patanjali, S. R., Simmons, A., Clines, G. A., Sartiel, A., Gatti, R. A., Chessa, L., Sanal, O., Lavin, M. F., Jaspers, N. G. J., Taylor, A. M. R., Arlett, C. F., Miki, T., Weissman, S. M., Lovett, M., Collins, F. S., and Shiloh, Y. (1995a). A single ataxia telangiectasia gene with a product similar to PI-3 kinase. *Science* 268: 1749-1753.
- Savitsky, K., Sfez, S., Tagle, D. A., Ziv, Y., Sartiel, A., Collins, F. S., Shiloh, Y., and Rotman, G. (1995b). The complete sequence of the coding region of the ATM gene reveals similarity to cell cycle regulators in different species. *Hum. Mol. Genet.* 4: 2025-2032.
- Shiloh, Y., Ziv, Y., Savitsky, K., Bar-Shira, A., Gilad, S., Vanagaite, L., Uchenik, V., Smith, S., Patanjali, S. R., Tagle, D., Simmons, A., Clines, G., Collins, F. S., Weissman, S., Lovett, M., and Rotman, G. (1994). Genetic, physical and functional analysis of the ataxia-telangiectasia locus on chromosome 11q22-23. 44th Annual Meeting of the American Society of Human Genetics, Montreal. *Am. J. Hum. Genet.* 55: A49.
- Shiloh, Y. (1995). Ataxia-telangiectasia: Closer to unraveling the mystery. *Eur. J. Hum. Genet.* 3: 116-138.
- Szpirer, C., Molne, M., Antonacci, R., Jenkins, N. A., Finelli, P., Szpirer, J., Riviere, M., Rocchi, M., Gilbert, D. J., Copeland, N. G., and Gallo, V. (1994). The genes encoding the glutamate receptor subunits KA1 and KA2 (GRIK4 and GRIK5) are located on separate chromosomes in human, mouse and rat. *Proc. Natl. Acad. Sci. USA* 91: 11849-11853.
- Thomas, J. E., Soriano, P., and Brugge, J. S. (1991). Phosphorylation of c-Src on tyrosine 527 by anchor protein tyrosine kinase. *Science* 254: 568-571.
- Uziel, T., Savitsky, K., Platzer, M., Ziv, Y., Helbitz, T., Rosenthal, A., Nehls, M., Boehm, T., Shiloh, Y., and Rotman, G. (1995). Genomic organization of the ATM gene. Submitted for publication.
- Zakian, V. A. (1995). ATM-related genes: What do they tell us about functions of the human gene. *Cell* 82: 685-687.