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Isolation of a mouse cDNA encoding MTJ1, a new murine member of the DnaJ family of proteins

(J domain; SEC63; heat-shock protein; chaperonin; Lewis lung carcinoma; *Saccharomyces cerevisiae*; *Escherichia coli*)

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SUMMARY

We report the isolation and sequencing of *MTJ1*, a 1792-bp cDNA from an M27 murine lung carcinoma cell line. The largest ORF within *MTJ1* encodes a 63 869-Da protein, containing a 73-amino-acid (aa) sequence (the J domain) that is conserved in proteins of the DnaJ family of chaperonins. The J domain of MTJ1 is bracketed by potential transmembrane domains in a similar configuration to the J domain of the yeast DnaJ-like protein, SEC63. Polyclonal antibodies raised against deduced aa sequences within MTJ1 recognized antigens of 62, 42 and 41 kDa that were enriched in the nuclear and heavy microsome subcellular fractions of murine tumor cells. Northern analysis detected a major 3.2-kb transcript that was present in all murine organs examined, but was relatively underexpressed in brain and heart.

INTRODUCTION

The term molecular chaperone designates proteins that promote the correct folding of other polypeptides, assembly and disassembly of protein complexes, and in eukaryotes the import of proteins into their target organelles (recently reviewed by Georgopoulos and Welch, 1993). Several chaperone proteins were initially identified as the products of HS genes, including the well-characterized DnaK and DnaJ chaperonins of *E. coli* (Goff and Goldberg, 1985; Liberek et al., 1988; Georgopoulos et al., 1990; Langer et al., 1992). In eukaryotes, a family of DnaK homologues (the Hsp70 family) and several DnaJ-like proteins have been described (Georgopoulos and Welch, 1993; Craig, 1990). DnaJ-like proteins have been shown to collaborate with members of the Hsp70 family in directing protein conformation and oligomerization in yeast (Silver and Way, 1993; Caplan et al., 1993; Mukai et al., 1994; Schwarz et al., 1994). In general, the aa sequences of DnaJ-like proteins are not as highly conserved as those of the Hsp70 family. However, all DnaJ-like proteins contain a well-conserved sequence of

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Abbreviations: aa, amino acid(s); Ab, antibody(ies); AP, affinity purified; bp, base pair(s); cDNA, DNA complementary to RNA; *dnaJ*, gene encoding the *E. coli* heat-shock protein DnaJ; ER, endoplasmic reticulum; *E.*, *Escherichia*; GCG, Genetics Computer Group (Madison, WI, USA); GST, glutathione *S*-transferase; HS, heat shock; Hsp70, 70-kDa HS protein; IgG, immunoglobulin G; kb, kilobase(s) or 1000 bp; *MTJ1*, gene encoding the murine DnaJ-like protein MTJ1; nt, nucleotide(s); ORF, open reading frame; p, plasmid; PA, polyacrylamide; pfu, plaque-forming units; PMSF, phenylmethylsulfonylfluoride; *S.*, *Saccharomyces*; SDS, sodium dodecyl sulfate; SEC63, *S. cerevisiae* DnaJ-like protein; TM, transmembrane (domain); *UTR*, untranslated region(s); YDJ1, *S. cerevisiae* DnaJ-like protein; [], denotes plasmid-carrier state; ′ (prime), denotes truncated gene at the indicated side.

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approx. 70 aa called the J domain, which is usually located at or near the N terminus (Caplan et al., 1993; Bork et al., 1992). The J domain is thought to be a site of functional interaction between DnaJ-like proteins and Hsp70 homologues (Liberek et al., 1991; Feldheim et al., 1992).

In this paper we describe the isolation of a cDNA using Ab that recognizes murine tumor cell proteins that were retained on a hydrophobic peptide (YVGVAPG) affinity column. This cDNA encodes a new member of the DnaJ family of proteins. To our knowledge, this is the first reported DnaJ-like protein to be isolated from a murine cell line. We propose to call this protein MTJ1, for murine tumor cell DnaJ-like protein 1.

EXPERIMENTAL AND DISCUSSION

(a) Isolation and sequence analysis of cDNA clones encoding MTJ1

An M27 Lewis lung carcinoma cDNA library was screened with an Ab (SF2A) generated against M27 cell proteins of 50–60 kDa that were retained on a hydrophobic peptide affinity column (YVGVAPG; Blood et al., 1988). Six plasmids containing cDNAs of similar size and identical restriction maps were isolated and the cDNA contained within pBSF was sequenced in its entirety (1792 bp; Fig. 1). The longest ORF in this cDNA (1659 bp) begins at nt 8 and predicts a protein of 63 869 Da. A potential polyadenylation signal, UCUAAA, occurs 22 nt upstream from a poly(A) tract at the 3′ end of the cDNA.

The deduced aa composition of the longest ORF indicates that it has a significant number of charged aa (16% D+E and 17% K+R) and a p*I* of 9.7. The predicted protein also contains one potential site for *N*-linked glycosylation at Asn^{475} and several potential sites for *O*-linked glycosylation and phosphorylation by protein kinases. Sequence comparisons revealed significant sequence similarity between aa 56–129 and the J domains of the *E. coli* DnaJ protein (38% identity; Bardwell et al., 1986) and several eukaryotic DnaJ-like proteins (43% and 41% identity with *S. cerevisiae* YDJ1 and SEC63, respectively; Caplan and Douglas, 1991; Feldheim et al., 1992; Fig. 2). These results indicate that the cDNA fragment contained in pBSF encodes a DnaJ-like protein which we propose to designate MTJ1. MTJ1 does not have significant sequence similarity to the *E. coli* DnaJ protein outside of the J domain.

Hydropathy analysis (Kyte and Doolittle, 1982) of the deduced aa sequence of MTJ1 suggests the presence of potential TM regions within aa 25–41 and within aa

```
                                                                    1
CAGCGCGATGTGGGTTCCCGGCTTCGGATCCGCGCGACTTCCCCAGCGCCGGCGCTCCGG      60
        M  W  V  P  G  F  G  S  A  R  L  P  Q  R  R  R  S  G      18
GTTGGAGTCATCCTCGGTGCGGCCGCTGTGGCTGCTGCTGCTGTTCCTGCTGGCAGCCGT     120
  L  E  S  S  S  V  R  P  L  W  L  L  L  L  F  L  L  A  A  V      38
GCGGCCAGTGCGCGCCTGGGAGAGCGGAGACCTGGAGTTGTTCGACTTGGTGGAAGAGGT     180
  R  P  V  R  A  W  E  S  G  D  L  E  L  F  D  L  V  E  E  V      58

GCAGTTGAACTTCTACGAGTTCCTCGGGGTGCAGCAGGATGCTTCATCTGCAGACATCAG     240
  Q  L  N  F  Y  E  F  L  G  V  Q  Q  D  A  S  S  A  D  I  R      78

AAAAGCATATCGTAAGCTTTCACTAACCTTACATCCAGACAAGAATAAAGATGAAAATGC     300
  K  A  Y  R  K  L  S  L  T  L  H  P  D  K  N  K  D  E  N  A      98

AGAAACTCAATTTAGACAATTGGTGGCCATTTATGAAGTTTTAAAGGATGATGAAAGAAG     360
  E  T  Q  F  R  Q  L  V  A  I  Y  E  V  L  K  D  D  E  R  R     118

GCAGAGGTATGATGATGTTCTGATCAATGGACTTCCAGATTGGCGACAGCCTGTATTCTA     420
  Q  R  Y  D  D  V  L  I  N  G  L  P  D  W  R  Q  P  V  F  Y     138

CTACAGGCGAGTGAGAAAAATGAGCAATGCTGAGCTGGCTTTGCTCTTGTTCATTATTCT     480
  Y  R  R  V  R  K  M  S  N  A  E  L  A  L  L  L  F  I  I  L     158
CACAGTGGGTCACTATGCTGTGGTTTGGTCCATCTACCTGGAAAAGCAACTGGATGAACT     540
  T  V  G  H  Y  A  V  V  W  S  I  Y  L  E  K  Q  L  D  E  L     178
GCTTGGTAGAAAAAAGAGAGAAAGGAAGAAGAAGACAGGAAGCAAGAGTGTGGACGCAGC     600
  L  G  R  K  K  R  E  R  K  K  K  T  G  S  K  S  V  D  A  A     198
AAAACTTGGTGCTTCTGAAAAGAACGAAAGATTGCTTATAAAACCACAATGGCATGATTT     660
  K  L  G  A  S  E  K  N  E  R  L  L  I  K  P  Q  W  H  D  L     218
GCTTCCATGCAAACTGGGGATTTGGTTTTGCCTTACACTAAAAGCATTGCCTCATCTAAT     720
  L  P  C  K  L  G  I  W  F  C  L  T  L  K  A  L  P  H  L  I     238
CCAGGATGCTGGGCAGTTTTATGCAAAATATAAGGAGACAAAATTGAAGGAAAAAGAAGA     780
  Q  D  A  G  Q  F  Y  A  K  Y  K  E  T  K  L  K  E  K  E  D     258
CGCACTGGCTAGAATTGAAATTGAAACCCTTCAAAAACAGAAGAAAGTTAAAGTTAAAAA     840
  A  L  A  R  I  E  I  E  T  L  Q  K  Q  K  K  V  K  V  K  K     278
ACCAAAACCAGAATTTCCTGTGTATATGCCTTTAGAAAATACGTATATTCAGTCTTATGA     900
  P  K  P  E  F  P  V  Y  M  P  L  E  N  T  Y  I  Q  S  Y  D     298
TCATGGAACTTCTATTGAAGAAATTGAGGAGCAAATGGATGACTGGCTGGAAAACAGGAA     960
  H  G  T  S  I  E  E  I  E  E  Q  M  D  D  W  L  E  N  R  K     318
AAGAACACAGAAAAGACAGGCACCTGAATGGACAGAAGAGGACCTCAGTCAACTGACAAG    1020
  R  T  Q  K  R  Q  A  P  E  W  T  E  E  D  L  S  Q  L  T  R     338
AAGTATGGTTAAGTTCCCAGGAGGGACTCCAGGTCGATGGGACAAGATTGCCCATGAATT    1080
  S  M  V  K  F  P  G  G  T  P  G  R  W  D  K  I  A  H  E  L     358
GGGTCGATCTGTGACAGATGTGACAACCAAAGCCAAGGAACTGAAGGACTCAGTCACCAG    1140
  G  R  S  V  T  D  V  T  T  K  A  K  E  L  K  D  S  V  T  S     378
CTCCCCCGGAATGACCAGGCTCTCTGAACTTAAGTCAAATGGTCAGAATTCCAGGCCAAT    1200
  S  P  G  M  T  R  L  S  E  L  K  S  N  G  Q  N  S  R  P  I     398
CAAGATAGCCACAGCCTTGCCAGATGACATCATCACGCAGCGGGAGGACTCAGCAGGGGC    1260
  K  I  A  T  A  L  P  D  D  I  I  T  Q  R  E  D  S  A  G  A     418
CATGGAGGATGAGGAGCACGAGGCTGCTGAGGCTGAGCAGGAGTCAGCGACCACAGAAGC    1320
  M  E  D  E  E  H  E  A  A  E  G  E  Q  E  S  A  T  T  E  A     438
CCGACCTCGGAGGCGGAAGTCAGCCAGGGCGGCCGAGGCTGTAACCAGAGTGGAGCCAGA    1380
  R  P  R  R  R  K  S  A  R  A  A  E  A  V  T  R  V  E  P  E     458
GGAGAAGCTGAGAGGAAAGAGGCAAAAAGACTTTGATATATCAGAACAAAACGATTCCAG    1440
  E  K  L  R  G  K  R  Q  K  D  F  D  I  S  E  Q  N  D  S  S     478
TGACGAAGAGAAACAACGAAAAGAAAGAACTCGTGCTGCAGAGGAGGCGTGGACTCAGAG    1500
  D  E  E  K  Q  R  K  E  R  T  R  A  A  E  E  A  W  T  Q  S     498
TCAGCAGAAACTTCTGGAATTGGCATTACAGCAATACCCTAAAGGAGCTTCTGACCGCTG    1560
  Q  Q  K  L  L  E  L  A  L  Q  Q  Y  P  K  G  A  S  D  R  W     518
GGACAAAATAGCCAAATGTGTCCCGTCTAAGAGTAAGGAAGACTGCATTGCTAGATACAA    1620
  D  K  I  A  K  C  V  P  S  K  S  K  E  D  C  I  A  R  Y  K     538
GCTGCTGGTTGAACTGGTCCAAAAGAAAAAGCAGGCTAAAAGCTGAATCTTCTGGAAGGT    1680
  L  L  V  E  L  V  Q  K  K  K  Q  A  K  S  *                   552
GACGATCAATGACCATCATTTTCCAAACAAGTACTCGAGAAATCTCATGCAGAAACTTAT    1740
ATTTTGTACCTCAGTATTTCTAAACATCATGTGCCTTAGTAAAAAAAAAAAA            1792
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Fig. 1. Nucleotide sequence of the *MTJ1* cDNA insert in pBSF (GenBank accession No. L16953) and deduced aa sequence. The J domain is underlined. **Methods:** The *MTJ1* cDNA was isolated from an M27 murine Lewis Lung carcinoma cDNA library constructed in the vector λZAPII (Stratagene, La Jolla, CA, USA) using a commercial kit (Invitrogen, San Diego, CA, USA). The library was screened with Ab SF2A (Gift from C. Blood, Schering-Plough Research Institute, Kenilworth, NJ, USA), according to the method of Sambrook et al. (1989). This Ab was originally generated against M27 cell proteins in the size range of 50–60 kDa that had been partially purified on an Affi-Gel affinity column conjugated with a synthetic peptide, YVGVAPG. Ab SF2A-positive cDNA clones were purified and subcloned into pBluescript SK(-) (Stratagene). The nt sequence of one positive clone, pBSF, was reconstructed from the overlapping sequences of exonuclease III-deleted subclones (Sambrook et al., 1989). Sequence analysis was carried out using the GCG sequence analysis software package version 7.

148–171. These regions are immediately N-terminal and immediately C-terminal to the J domain, respectively (Fig. 3). This arrangement of potential TM regions on either side of the J domain is strikingly similar to that predicted for the *S. cerevisiae* DnaJ-like protein SEC63 (Feldheim et al., 1992; Fig. 3). The TM regions of SEC63 anchor it in the ER membrane and project the J domain into the ER lumen, where it interacts with BiP (Kar2p), an ER-specific Hsp70 homologue.

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ECDJ     (1) MAKQ.DY YE I LGV SKT A EER EI RK AY KRLAMK YHPD RNQGDKEAEAK..... F KEIKEA YE V L T D SQK R AA Y DQYGEAAF  (74)
YDJ1     (1) MVKETKF YD I LGV PVT A TDV EI KK AY RKCALK YHPD KNPSE.EAAEK..... F KEASAA YE I L S D PEK R DI Y DQFGEDGL  (74)
MTJ1    (56) EEVQLNF YE F LGV QQD A SSA DI RK AY RKLSLT LHPD KNKDE.NAETQ..... F RQLVAI YE V L K D DER R QR Y DDVLINGL (129)
SEC63  (120) ATKLFDP YE I LGI STS A SDR DI KS AY RKLSVK FHPD KLAKGLTPDEKSVMEET Y VQITKA YE S L T D ELV R QN Y LKYGHPDG (200)
```

Fig. 2. Alignment of J domains of ECDJ (*E. coli* DnaJ; Bardwell et al., 1986), YDJ1 (*S. cerevisiae*; Caplan and Douglas, 1991), MTJ1 and SEC63 (*S. cerevisiae*; Feldheim et al., 1992). Sequence alignments were carried out using the GCG sequence analysis software package version 7 and the accompanying databases. The positions of the first and last aa used in the alignment are in parentheses. Identical or conservatively substituted aa (four out of four) are boxed.

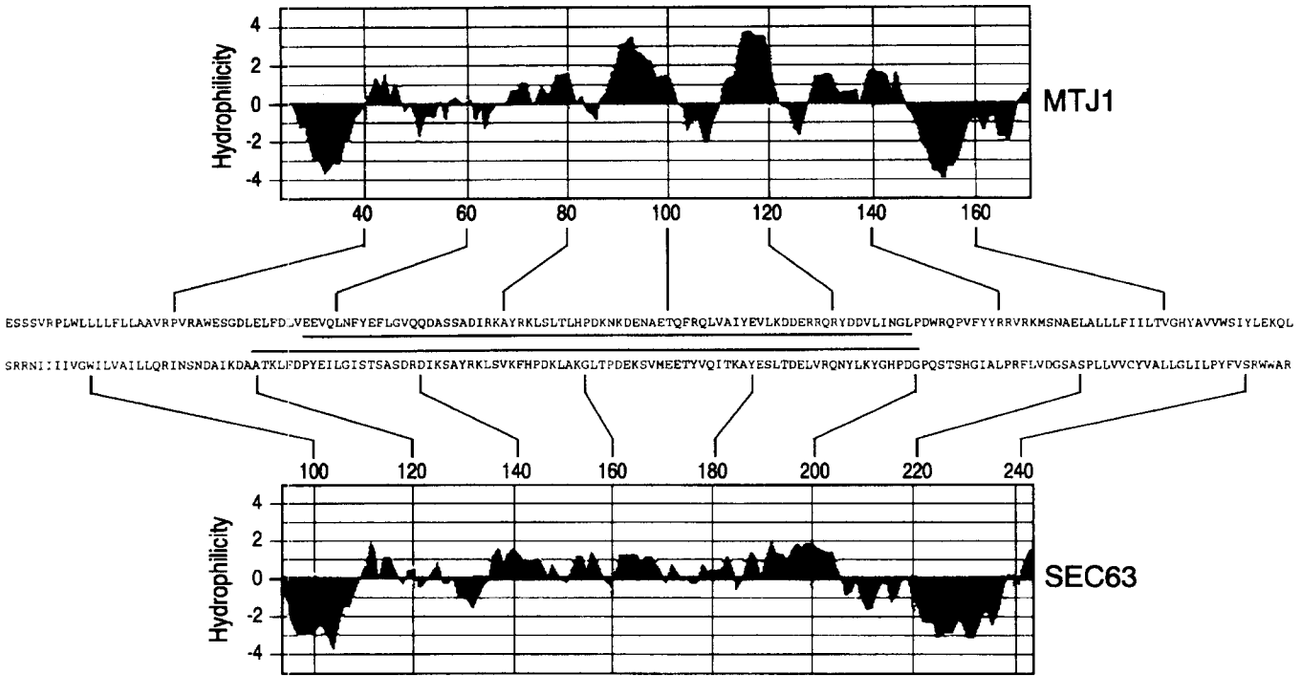

Fig. 3. Comparison of the hydropathy plots (Kyte and Doolittle, 1982) of the J domains (underlined) and bracketing aa sequences of MTJ1 and SEC63. The window size chosen was 7 aa. The aa residue number and the accompanying aa regions are shown either below (MTJ1) or above (SEC63) the corresponding hydropathy plots.

(b) Size and subcellular localization of MTJ1

To investigate the size and subcellular localization of MTJ1 in murine cell lines, polyclonal Ab were raised against (*i*) a synthetic peptide representing the C-terminal tail of MTJ1 (aa 542–552; Ab 27), and (*ii*) a GST:MTJ1$^{146-552}$ fusion protein (Ab D). AP Ab 27 and AP Ab D detect a 62-kDa protein in extracts of M27 lung carcinoma, B16F10 melanoma and 3T3 fibroblasts (Fig. 4; B16F10 data). This protein is enriched in the heavy microsome and nuclear fractions of both B16F10 and M27 cells (Fig. 4; B16F10 data). AP Ab D also recognizes proteins of 41 and 42 kDa that are enriched in the nuclear and heavy microsome fractions, respectively (Fig. 4). The 62, 42 and 41-kDa proteins are not recognized by pre-immune sera (data not shown). The similarity in size between the 62-kDa protein and the predicted size for MTJ1, and the finding that the 62-kDa protein is recognized by both Ab suggests that it may represent the product of translation from the start codon located at nt 8 in the *MTJ1* cDNA. The 41/42-kDa proteins may represent alternative translation products from an internal, in-frame start site. We have found that lysates of *E. coli*[pBSF] produce one recombinant protein of 42 kDa that is recognized by Ab 27, Ab D and the original Ab SF2A (data not shown). Since the start codon at nt 8 of *MTJ1* is not associated with a ribosome-binding site in pBSF (Fig. 1), the recombinant protein is likely to represent translation of the ORF beginning at nt 440. The size of this internal ORF predicts a protein of 46 831 Da, and does not include the J domain. We are currently investigating the relationship between the murine antigens recognized by the anti-MTJ1 Ab and the predicted products of the cDNA.

(c) Northern analysis

Northern analysis using poly(A)$^+$RNA extracted from M27 cells (data not shown) and murine organs detected a major transcript of 3.2 kb (Fig. 5A). Since the size of one of the proteins detected by Western analysis corresponded closely with the predicted size of MTJ1, a complete ORF encoding MTJ1 may be contained on the cDNA fragment in pBSF. If so, the short 5′-*UTR* indi-

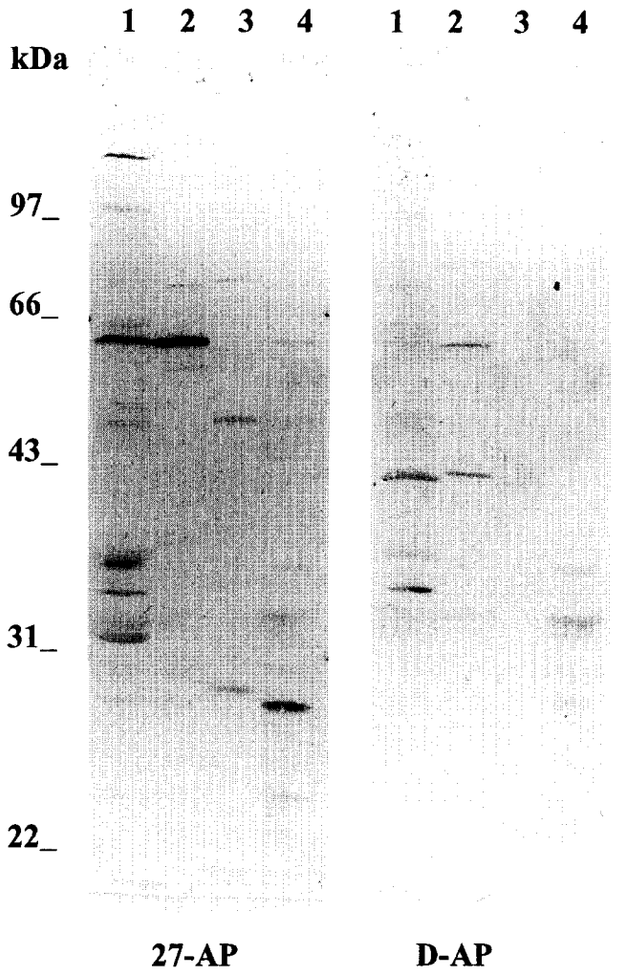

Fig. 4. Immunoblotting of B16F10 subcellular fractions with anti-MTJ1 Ab. Duplicate protein samples (30 μg) representing nuclei (lane 1), heavy microsomes (lane 2), cytosol (lane 3) and light microsomes (lane 4) were electrophoresed, transferred to nitrocellulose, and probed with AP Ab 27 (27-AP) or AP Ab D (D-AP). **Methods:** For Ab 27, rabbits were immunized with a synthetic peptide representing MTJ1 aa 542–552 conjugated to keyhole limpet hemocyanin. Ab 27 IgG was harvested on protein A-agarose (Boehringer-Mannheim, Indianapolis, IN, USA; Harlow and Lane, 1988), and then affinity purified on a column prepared by coupling 2 mg synthetic peptide to 2 ml SulfoLink coupling gel (Pierce, Rockford, IL, USA) according to the manufacturer's instructions. Ab 27 IgG (5 mg) was applied to the affinity column in 10 mM Tris·HCl pH 8.0 (CB). The flow-through was collected and re-applied to the column twice, the column was washed with 10 vols. CB, and the AP Ab was eluted with 100 mM glycine pH 3.0 (EB) and neutralized. For Ab D, a GST:MTJ1 fusion protein was prepared by subcloning the *MTJ1* nt sequence encoding aa 146–552 in-frame with the GST gene in the vector pGEX3X (Pharmacia, Piscataway, NJ, USA). Purification of the GST:MTJ1 fusion protein from bacterial lysates was accomplished by affinity chromatography on glutathione-Sepharose (Pharmacia) according to the manufacturer's instructions, followed by gel electrophoresis. The IgG fraction of Ab D was AP on recombinant MTJ1 protein as follows. λZAP II phage containing the *MTJ1* insert were plated on NZY agarose (2000 pfu/plate), overlaid with IPTG-impregnated filters, and grown at 37°C overnight. The filters were then washed in TNT (10 mM Tris·HCl pH 8.0/150 mM NaCl/0.05% Tween-20), blocked for 30 min in 5% non-fat dry milk in TNT, and then incubated with Ab D IgG overnight at 4°C (6 mg Ab in 60 ml TNT containing 0.5 mg/ml Triton X-100 lysate of *E. coli*[pGEX3X]). Bound Ab was eluted with EB and neutralized. Subcellular fractionation was accomplished by a modification of the method of Brigstock et al. (1991). Cells were harvested from confluent plates with Versene 1:5000 (Gibco-BRL, Gaithersburg, MD, USA), counted, and resuspended to 10^8/5 ml in H-buffer (10 mM Tris·HCl pH 7.5/2 mM $MgCl_2$/80 mM sucrose/2 mM EGTA/1 mM PMSF/2 μg aprotinin per ml). After 10 min on ice, the cells were lysed in a Dounce homogenizer, centrifuged ($800 \times g$ for 10 min), resuspended in fresh H-buffer, re-homogenized and pelleted again. Nuclei were further purified from the $800 \times g$ pellet by the method of Muramatsu et al. (1974), and resuspended in S-buffer (10 mM Tris·HCl pH 7.5/0.25 M sucrose/1 mM PMSF/2 μg aprotinin per ml). The two $800 \times g$ supernatants were combined, stabilized with sucrose (to 0.25 M), and subjected to sequential centrifugations to produce the heavy and light microsome fractions ($10\,000 \times g$ for 20 min and $100\,000 \times g$ for 90 min, respectively). The microsome pellets were resuspended in S-buffer and the final $100\,000 \times g$ supernatant, containing the soluble cytosol, was concentrated 5–10 fold in Centricon microconcentrators (Amicon, Beverly, MA, USA; molecular weight cut-off of 3000). Subcellular fraction proteins were analysed by 0.1% SDS-10% PAGE, transferred to nitrocellulose, blocked with 5% non-fat dry milk in TNT (30 min, 22°C), and incubated with either 27-AP or D-AP (10 μg/ml; 90 min at 22°C). Following three 10-min washes in TNT, the blots were incubated with alkaline phosphatase-conjugated goat anti-rabbit IgG (Promega, Madison, WI, USA; 1:5000 in TNT; 60 min at 22°C), washed 3×10 min in TNT, and developed in 100 mM Tris·HCl pH 9.5/100 mM NaCl/5 mM $MgCl_2$ containing 330 μg/ml nitro blue tetrazolium and 165 μg/ml 5-bromo-4-chloro-3-indolyl phosphate.

cates that the cDNA fragment was 5′ truncated, and the full-length transcript would have a lengthy 5′-*UTR*. A minor 6.5-kb transcript was also detected in M27 cells and murine organs and may be the product of an alternative splicing event.

To determine whether there is any tissue specificity in the expression of the transcripts, poly(A)$^+$RNAs from six different adult murine organs, including lung, liver, brain, spleen, kidney and heart, were examined by Northern analysis. Both the 3.2-and 6.5-kb messages were detectable in all six organs (Fig. 5A). For the purpose of comparison, steady-state message levels in each organ were quantitated by PhosphorImager analysis and expressed as a percentage of the message level in the lung (Fig. 5B and C). Steady-state levels of both the 3.2-kb and the 6.5-kb transcripts were relatively low in brain and heart. The quantitative analysis also revealed that the 3.2- and 6.5-kb messages exhibit different patterns of expression in these tissues.

(d) Conclusions

(*1*) We report the isolation of a cDNA clone encoding MTJ1, the first DnaJ-like protein to be isolated from a murine cell line.

(*2*) The deduced aa sequence for MTJ1 contained a J domain bracketed by potential TM regions similar to that found for the yeast DnaJ-like protein SEC63.

(*3*) Western blot analysis of murine tumor cell extracts

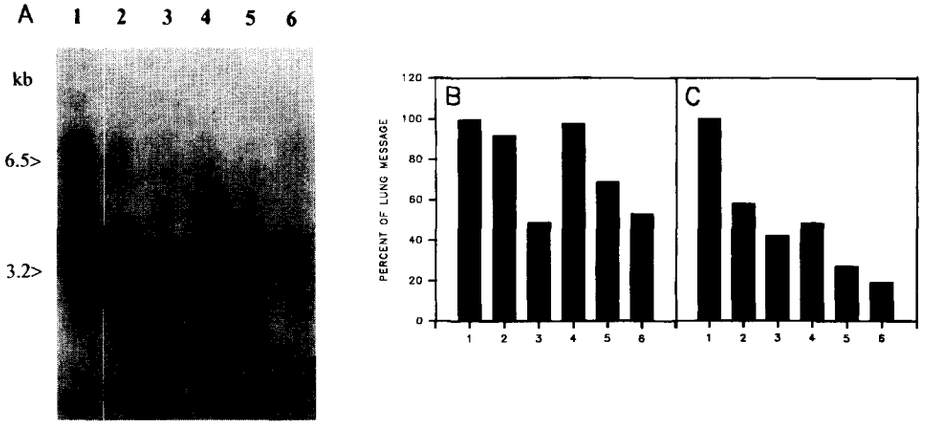

Fig. 5. Northern analysis of *MTJ1* transcripts. (**A**) *MTJ1* transcripts expressed in different mouse organs. **1**, lung; **2**, liver; **3**, brain; **4**, spleen; **5** kidney; **6**, heart. The major 3.2-kb transcript and minor 6.5-kb transcript are indicated by arrowheads. (**B**) Quantitation of 3.2-kb message levels by PhosphorImager analysis. (**C**) Quantitation of 6.5-kb message levels by PhosphorImager analysis. **Methods:** Poly(A)$^+$RNA (1 μg) from each organ (Davis et al., 1986; MacDonald et al., 1987) was resolved on a 0.8% agarose-2.2 M formaldehyde gel, transferred to nitrocellulose, and probed with the ^{32}P-labelled 1.3-kb *Pst*I fragment of *MTJ1* (Sambrook et al., 1989). For quantitation of the signal intensities, the Northern blot was scanned using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA, USA) and the results were quantified using the Molecular Dynamics ImageQuant program. The energy represented in each band was quantitated by defining a rectangular area around the band and integrating the signal within the volume of that rectangle. All the bands were quantitated using the same rectangular area, as was the background measurement that was subtracted from each band value. The energy associated with each 3.2- and 6.5-kb band is expressed as a percentage of the 3.2- and 6.5-kb band intensities for lung poly(A)$^+$RNA, respectively.

with Ab raised against the deduced aa sequence of MTJ1 detected major antigens of 62, 42 and 41 kDa. These antigens were enriched in the nuclear and heavy microsome fractions of murine tumor cells.

(*4*) Northern analysis detected a full length transcript for *MTJ1* of at least 3.2-kb that was differentially expressed in mouse organ tissues.

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