

# Two Novel Mouse Genes—*Nubp2*, Mapped to the *t*-Complex on Chromosome 17, and *Nubp1*, Mapped to Chromosome 16—Establish a New Gene Family of Nucleotide-Binding Proteins in Eukaryotes

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**Two novel mouse genes and one novel human gene that define distinctive eukaryotic nucleotide-binding proteins (NUBP) and are related to the mrp gene of prokaryotes are characterized. Phylogenetic analyses of the genes, encoding a short form (*Nubp2*) and a long form (*Nubp1*) of NUBP, clearly establish them as a new NUBP/MRP gene family that is well conserved throughout phylogeny. In addition to conserved ATP/GTP-binding motifs A (P-loop) and A', members of this family share at least two highly conserved sequence motifs, NUBP/MRP motifs  $\alpha$  and  $\beta$ . Only one type of NUBP/MRP gene has been observed thus far in prokaryotes, but there are two types in eukaryotes. One group includes mouse *Nubp1*, human *NBP*, yeast *NBP35*, and *Caenorhabditis elegans* F10G8.6 and is characterized by a unique N-terminal sequence with four cysteine residues that is lacking in the other group, which includes mouse *Nubp2*, human *NUBP2*, and yeast *YIA3w*. Northern blot analyses of the two mouse genes show distinctive patterns consistent with this classification. Mouse *Nubp2* is mapped to the *t*-complex region of mouse Chromosome 17, whereas *Nubp1* is mapped to the proximal region of mouse Chromosome 16. Interestingly, both regions are syntenic with human chromosome 16p13.1–p13.3, suggesting that a chromosomal breakage between *Nubp2* and *Nubp1* probably**

**occurred during the evolution of mouse chromosomes.** © 1999 Academic Press

## INTRODUCTION

Many proteins function by drawing on energy from the hydrolysis of ATP/GTP. The ATPase/GTPase superfamily is thereby involved in signal transduction, mechanical movement, and transport of molecules. Many, though not all, of these proteins share a characteristic sequence motif “[GA]-X<sub>2</sub>-(G)-X-G-K-[ST],” called the “phosphate-binding loop (P-loop)” (Walker *et al.*, 1982; Saraste *et al.*, 1990). Refined analyses have identified a subfamily, the “partitioning ATPase gene family” (Koonin, 1993). This subfamily includes the *Escherichia coli* *MinD* gene, a membrane-associated ATPase that inhibits cell division at the poles and consequently induces normal cell division (de Boer *et al.*, 1991), and based on sequence similarity, the *E. coli* *mrp* gene (Dardel *et al.*, 1990).

One member of the partitioning ATPase gene family in human has been cloned. Based on its ATP/GTP-binding motif, this gene was called nucleotide-binding protein (*NBP*) (Shahrestanifar *et al.*, 1994). Sequence similarity searches showed a likely relationship to the bacterial *MinD* gene. The yeast (*Saccharomyces cerevisiae*) homolog of human *NBP* has also been cloned and characterized as *NBP35*, encoding a protein of 35 kDa (Vitale *et al.*, 1996). Complementation analyses using yeast knockouts revealed that a cysteine-rich N-terminal domain, which is conserved in the human *NBP*, is indispensable for the function of the gene (Vitale *et al.*, 1996). Another yeast gene, *YIA3w*, that encodes a 31.9-kDa protein was also predicted from the yeast genome sequencing project (Voss *et al.*, 1995). The putative *YIA3w* shows weak similarity to the previously discovered human *NBP* and yeast *NBP35* genes, but lacks

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the cysteine-rich N-terminal region common to those genes. The authors put this gene in the MinD/Mrp family based on sequence similarity to *E. coli* MinD. Further analysis has not been reported, however, and there has been considerable confusion about the interrelationships and categorization of this cohort of nucleotide-binding proteins.

Recently, through a sequencing and mapping project of a mouse 7.5-day postconception (dpc) ectoplacental cone cDNA library, 10 novel genes were mapped to the proximal region of Chromosome 17, a region best known as the *t*-complex (Ko *et al.*, 1998). One of the clustered genes shows significant similarity to human NBP, yeast NBP35, and yeast YIA3w. Because this mouse gene does not have the cysteine-rich N-terminal domain, which is common to both human NBP and yeast NBP35, we speculated that there might be another mouse gene that contains this cysteine-rich N-terminal domain. The existence of this gene has also been confirmed, and we describe here the isolation and characterization of the two mouse genes, termed nucleotide-binding protein 2 (*Nubp2*) and nucleotide-binding protein 1 (*Nubp1*). We also present data on the sequencing and characterization of the human ortholog of mouse *Nubp2*. Based on the phylogenetic analyses of these newly isolated genes along with the previously identified genes, we propose that this NUBP/MRP gene family forms a new subfamily of ATP/GTP-binding proteins, which is clearly distinguishable from the *MinD* gene family. We also propose that this new gene family includes two clearly distinguishable subtypes in eukaryotes, with only one closely related gene, *mrp*, in prokaryotes.

## MATERIALS AND METHODS

**Cloning and sequence analyses of cDNAs.** Construction of the mouse 7.5-dpc ectoplacental cone cDNA library and a large-scale cDNA sequencing and mapping project are described (Ko *et al.*, 1998). As a result, 10 of 155 unique clones were mapped to the *t*-complex of Chromosome 17. One of those clones, C0001C09 (gene name: *D17Wsu11e*), which mapped to the same bin as *D17Mit55*, was used for this study. This cDNA was renamed nucleotide-binding protein 2 (*Nubp2*). The cDNA sequence for mouse *Nubp1* was identified through the BlastN search against the dbEST database at NCBI/NIH (Boguski *et al.*, 1993). The corresponding cDNA clones carrying the BALB/c mouse lung EST (Clone ID 695233) from the IMAGE Consortium were purchased from Research Genetics (Lennon *et al.*, 1996). For the human *NUBP2* gene, the corresponding cDNA clone was sought on the expressed sequence tag database (dbEST) (Boguski *et al.*, 1993) at NCBI/NIH, and the IMAGE clone H17161 (Lennon *et al.*, 1996) was purchased from Research Genetics. Since the cDNA clone did not carry the entire coding sequence, a human teratocarcinoma cDNA library (Skowronski *et al.*, 1988) was screened with this human cDNA clone as a probe as described (D'Esposito *et al.*, 1994).

These cDNA clones were completely sequenced with *Taq* DyeDeoxy Terminators on an ABI 377 automated sequencer (Perkin-Elmer). DNA sequence analyses were performed using the MacDNASIS program (Hitachi). Multiple alignment was performed with the Higgins algorithm (Higgins and Sharp, 1988). Similarity searches of nucleotide and amino acid sequence were performed by the Blast server at NCBI/NIH (Altschul *et al.*, 1990).

The GenBank accession numbers for these sequences are AF114169 (*Nubp2*), AF114170 (*Nubp1*), and AF118394 (*NUBP2*).

**Northern blot.** Membranes were purchased from Clontech. The membrane was prehybridized in ExpressHyb solution (Clontech) for 30 min at 68°C. Hybridization was performed for 1 h at 68°C with a random-primed [ $\alpha$ -<sup>32</sup>P]dCTP (Amersham)-labeled DNA probe. The membrane was washed under high-stringency conditions: twice in 2× SSC at room temperature for 5 min and then twice in 0.1× SSC and 0.1% SDS at 68°C for 30 min.

**Mapping of *Nubp1* on mouse chromosome 16.** The mapping of the mouse *Nubp1* gene was performed according to the previously described method (Ko *et al.*, 1994). The PCR primer pair was designed from the 3'-untranslated region (UTR) of the cDNA to obtain the sequence polymorphism between C57BL/6J and *Mus spretus* (Takahashi and Ko, 1993). The primer pairs were *Nubp1*-up6, 5'-CAGAG-GATCCGAGACTTTTGTAA-3', and *Nubp1*-dn6, 5'-CGCCCGTAC-CTTTGATCTC-3'. The PCR heteroduplex was used to type the genomic DNAs of The Jackson Laboratory BSS Backcross Mouse Panels (Rowe *et al.*, 1994). The genotypes were scored by visual inspection of the heteroduplex bands and analyzed by the Map Manager program (Manly, 1993). The raw mapping data for the cross are accessible through The Jackson Laboratory (<http://www.jax.org/resources/documents/cmdata/BSS.html>). Corresponding segments of the human genome were sought using the Human/Mouse Homology Relationships (DeBry and Seldin, 1996; NCBI: <http://www.ncbi.nlm.nih.gov/Homology/>).

## RESULTS AND DISCUSSION

### *Isolation and Characterization of Mouse Nubp2*

The 1.4-kb insert of cDNA clone C0001C09 was completely sequenced (Fig. 1). The sequence next to the putative initiation codon, AGCGAAatgG, is consistent with Kozak's (1996) consensus sequence in a strong context. A variant form of a poly(A) signal, TATAAA, is then located at the 3'-end of the cDNA. An open reading frame (ORF) of 275 amino acid residues terminates at a TGA stop codon at nucleotide 848 (asterisk in Fig. 1). We infer that the clone C0001C09 includes a complete transcription unit, with a full ORF and 3'-UTR.

Amino acid homology searches by BlastP (Altschul *et al.*, 1990) revealed that this gene is apparently novel and has a strong similarity to the predicted yeast *YIA3w* gene (Voss *et al.*, 1995), yeast *NBP35* (Vitale *et al.*, 1996), human *NBP* (Shahrestanifar *et al.*, 1994), and *Caenorhabditis elegans* ORF F10G8.6 (Wilson *et al.*, 1994), in that order. Weak similarity was also found to *mrp* genes from various prokaryotes, including *Methanococcus jannaschii*, *Synechocystis* sp., *Haemophilus influenzae*, *Pseudomonas fragi*, *E. coli*, *Bacillus subtilis* and *Mycobacterium replae*. The *mrp* gene, which is named after the adjacent gene "methionyl-tRNA synthetase (*metG*)," was originally identified as an *E. coli* ATPase of unknown function (Dardel *et al.*, 1990). The gene also showed some similarity to prokaryote proteins that help to promote proper cell division, *Me. jannaschii* and *E. coli* *MinD*.

We originally named this novel mouse gene nucleotide-binding protein short form (*Nbps*), because this gene, like yeast *YIA3w*, lacks the longer N-terminal, including the C-X13-C-X2-C-X5-C motif that is common among NBP, F10G8.6, and NBP35 (Vitale *et al.*,

G	CGA	AAA	GCA	ACT	AGT	AGC	GGA	<u>ATG</u>	GAG	GCT	GCT	GCC	GGT	GAG	CGT	GCA	GAA	CCC	GGG	58
								M	E	A	A	A	G	E	R	A	E	P	G	12
AAC	CTG	GCC	GGG	GTG	CGA	CAC	ATC	ATT	CTT	CTT	TCT	GGA	AAG	GGT	GGC	GTT	GGG	AAA	118	
N	L	A	G	V	R	H	I	I	L	<b>V</b>	<b>L</b>	<b>S</b>	<b>G</b>	<b>K</b>	<b>G</b>	<b>G</b>	<b>V</b>	<b>G</b>	<b>K</b>	32
AGC	ACC	ATC	TCC	ACG	GAG	TTG	GCC	CTG	GCT	CTG	CGC	CAC	CAG	GGC	AAG	AAG	GTG	GGA	ATC	178
<b>S</b>	<b>T</b>	<b>I</b>	<b>S</b>	<b>T</b>	<b>E</b>	<b>L</b>	<b>A</b>	<b>L</b>	<b>A</b>	<b>L</b>	<b>R</b>	<b>H</b>	<b>Q</b>	<b>G</b>	<b>K</b>	<b>K</b>	<b>V</b>	<b>G</b>	<b>I</b>	52
CTA	GAT	GTG	GAC	CTG	TGC	GGT	CCC	AGC	ATA	CCA	CAC	ATG	CTC	CGT	GCA	CAA	GGA	AAG	GCC	238
L	<b>D</b>	<b>V</b>	<b>D</b>	<b>L</b>	<b>C</b>	<b>G</b>	P	S	I	P	H	M	L	R	A	Q	G	K	A	72
GTG	CAC	CAG	TGT	GAC	AAT	GGC	TGG	GTG	CCT	GTC	TTT	GTG	GAT	CAG	GAG	CAG	AGC	ATC	TCC	298
V	H	Q	C	D	N	G	W	V	P	V	F	V	D	Q	E	Q	S	I	S	92
CTT	ATG	TCT	GTG	GGG	TTC	CTG	CTG	GAA	AAC	CCT	GAT	GAG	GCC	GTG	GTG	TGG	AGA	GGT	CCC	358
L	M	S	V	G	F	L	L	E	N	P	D	E	A	V	<b>V</b>	<b>W</b>	<b>R</b>	<b>G</b>	<b>P</b>	112
AAG	AAA	CAT	GCA	CTG	ATA	AAG	CAG	TTT	GTG	TCT	GAC	GTG	GCC	TGG	GGG	CAG	CTG	GAT	TAT	418
<b>K</b>	<b>K</b>	<b>H</b>	<b>A</b>	<b>L</b>	<b>I</b>	<b>K</b>	<b>Q</b>	<b>F</b>	<b>V</b>	<b>S</b>	<b>D</b>	<b>V</b>	<b>A</b>	<b>W</b>	<b>G</b>	<b>Q</b>	<b>L</b>	<b>D</b>	<b>Y</b>	132
CTG	GTT	GTG	GAC	ACA	CCT	CCA	GGG	ACC	TCT	GAT	GAG	CAC	ATG	GCC	ACT	ATG	GAA	GCC	CTG	478
<b>L</b>	<b>V</b>	<b>V</b>	<b>D</b>	<b>T</b>	<b>P</b>	<b>P</b>	<b>G</b>	<b>T</b>	<b>S</b>	<b>D</b>	E	H	M	A	T	M	E	A	L	152
CGC	CCC	TAC	AGG	CCC	CTT	GGG	GCT	CTT	GTA	GTC	ACC	ACA	CCA	CAG	GCG	GTG	TCT	ATT	GGG	538
R	P	Y	R	P	L	G	A	L	V	V	T	T	P	Q	A	V	S	I	G	172
GAT	GTG	AGG	CGG	GAG	CTG	ACC	TTC	TGT	AAG	AAG	ACT	GGG	CTG	CAG	GTG	ATA	GSS	GTC	ATA	598
D	V	R	R	E	L	T	F	C	K	K	T	G	L	Q	V	<u>I</u>	<u>G</u>	<u>V</u>	<u>I</u>	192
GAG	AAC	ATG	AGC	GGC	TTC	ACC	TGC	CCA	CAC	TGC	GCT	GAG	TGC	ACC	AAT	GTC	TTC	TCC	AGC	658
<u>E</u>	<u>N</u>	<u>M</u>	<u>S</u>	G	F	T	C	P	H	C	A	E	C	T	N	V	F	S	S	212
GGC	AGT	GGG	GAG	GAG	CTG	GCC	CGG	CTG	GCT	GGA	GTT	CCC	TTT	TTA	GGC	TCT	GTC	CCC	CTG	718
G	S	G	E	E	L	A	R	L	A	G	V	P	F	L	G	S	V	P	L	232
GAT	TCC	CAA	CTT	ACC	AGG	AGC	TTG	GAG	GAA	GGG	CGT	GAC	TTC	ATC	CAG	GAA	TTT	CCC	AAA	778
D	S	Q	L	T	R	S	L	E	E	G	R	D	F	I	Q	E	F	P	K	252
AGC	ACT	GCA	TAT	TCC	GCA	CTC	ACG	TCC	ATA	GCC	CAG	AGA	GTT	GTG	CAC	AGG	ATG	TCT	GCC	838
S	T	A	Y	S	A	L	T	S	I	A	Q	R	V	V	H	R	M	S	A	272
CTG	TGC	TCC	<u>TGA</u>	CCG	CCT	GGC	AGC	CTT	CTG	GTG	CTG	CAT	GCC	TGT	AGG	ACC	TTT	GGG	AGC	898
L	C	S	*																	275
AGA	GGC	CCT	CAG	GAC	TGT	TTT	GGG	ACC	TGT	AGT	TGT	TGG	CCT	GGG	CAA	TGG	CTG	GAC	TAT	958
GCG	ATT	ACA	TCC	ATG	CAG	AGA	TCT	GCA	GGC	CCC	CAT	GTC	CTT	GGC	TGA	CAC	TAG	CTT	CAC	1018
CAA	ACT	AGT	CTA	TAG	CTG	AGG	GGA	TAG	CCT	GCG	TCC	TGA	TTC	CTT	GAG	ATA	ATA	GCA	GCC	1078
TTA	ACC	TTT	AGC	CTA	TAG	GCT	TTC	GTC	CAG	CCC	TAG	TCA	CTT	CTG	CCC	CAG	GTC	AAC	AGG	1138
CTG	CTC	TCA	TAG	CCA	CAC	CAC	ACA	GGA	GCT	GGC	CAG	TGC	TTT	CTC	ATC	GAG	ACT	GAC	CCA	1198
CAC	ATG	CTG	TGT	TGT	GTG	CCT	TTT	GTG	ACA	CTG	TGG	TTT	ACC	GGC	ACA	TCT	GGT	AAC	TTT	1258
ACA	GAA	CTC	TGC	TAT	TAA	ACA	TAC	CGC	CCC	GTC	TTA	GGA	AGG	ACC	CAG	CAT	CCA	AGC	CCT	1318
TTG	TCC	TCA	GCG	AGG	TGA	CTC	TTG	ATT	CCC	CTA	CAT	TTC	ATG	TCT	GGA	ACA	AGC	AGT	ATT	1378
AGT	AAA	GAT	<u>TAT</u>	<u>AAA</u>	GGG	CCT	G													1400

**FIG. 1.** Mouse *Nubp2* nucleotide and amino acid sequence (clone C0001C09). Start codon is double-underlined; ATP/GTP binding motif "A" (P-loop) is in boldface type; motif  $\alpha$  is outlined; NUBP/MRP consensus pattern is italicized and in boldface type; motif  $\beta$  is wavy underlined; stop codon is underlined; poly(A) signal is dashed underlined.

1996). However, based on the recommendation by the Nomenclature Committee, the gene was renamed nucleotide-binding protein 2 (*Nubp2*) to avoid confusion with another unrelated yeast gene Nap1-binding protein (*NBP2*) (GenBank Accession No. YSCNBP2). Therefore, the root symbol for this family has been changed from NBP to NUBP.

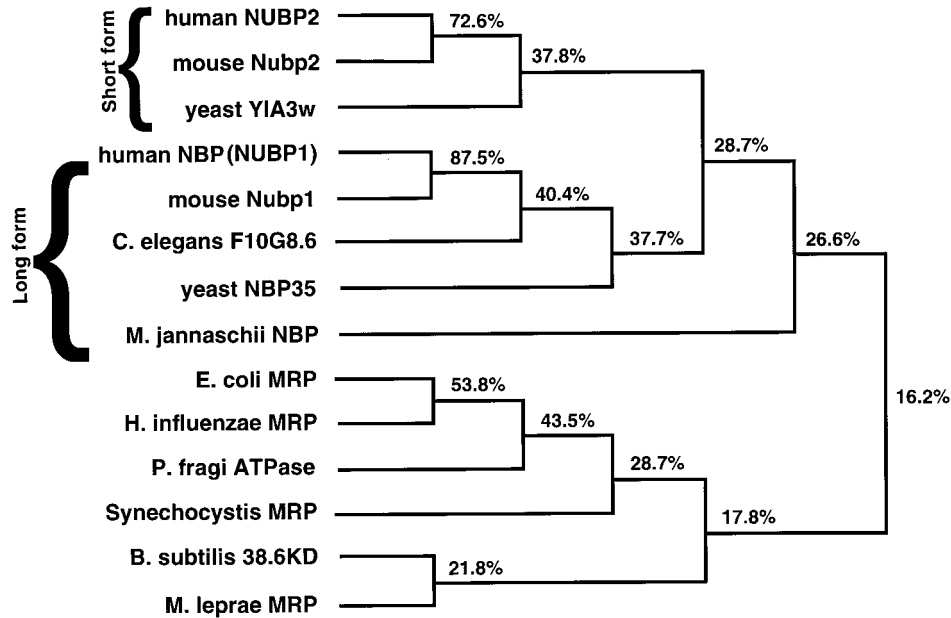
#### Isolation and Characterization of Mouse *Nubp1*

Unlike *Nubp2*, the reported human *NBP* gene has a cysteine-rich N-terminal sequence. This prompted a search for a possible "longer" mouse *NBP* gene. A BlastN search with the human *NBP* sequence against the public dbEST (NCBI) (Boguski *et al.*, 1993) identified two mouse ESTs from the IMAGE Consortium collections (Lennon *et al.*, 1996). The 1.3-kb insert of the cDNA clone was completely sequenced (data not shown). The cDNA has a translation initiation site that matches the Kozak (1996)

consensus sequence in a strong context, and a canonical poly(A) signal AATAAA was found at the 3'-end of the cDNA. An ORF of 320 amino acid residues terminates at a TGA stop codon at nucleotide 977. We infer that the clone includes a complete transcription unit, with a full ORF and 3'-UTR. Overall, the protein is 45 amino acids longer than *Nubp2*. As expected, the protein showed highest similarity to the human NBP (87.5%) and contains the cysteine-rich N-terminal sequence, including the C-X13-C-X2-C-X5-C motif. Therefore, we named this novel mouse gene *Nubp1* (nucleotide-binding protein 1).

#### Isolation and Characterization of Human *NUBP2*

To complete the putative repertoire of two types of NUBP in human and mouse, a human ortholog of mouse *Nubp2* was sought. Sequence similarity searches against the public EST database (dbEST, NCBI) (Boguski *et al.*, 1993) showed significant hits in



**FIG. 2.** Phylogenetic tree of NUBP/MRP subfamily members. Percentage protein similarity is indicated to the right of each branch point of the tree. Amino acid sequences were aligned to produce the tree using the Higgins algorithm with the MacDNASIS program (Hitachi). The names of genes, their accession numbers, and their references are as follows: human *NUBP2* (nucleotide-binding protein 2, AF118394, this paper); mouse *Nubp2* (nucleotide-binding protein 2, AF114169, this paper); yeast *YIA3w* (YIA3\_ yeast hypothetical 31.9 KD protein in BET1-PAN1, P40558 (Voss *et al.*, 1995)); human *NBP* (nucleotide-binding protein, U01833 (Shahrestanifar *et al.*, 1994)); mouse *Nubp1* (nucleotide-binding protein 1, AF114170, this paper); *C. elegans* F10G8.6 (hypothetical protein, Z80216 (Wilson *et al.*, 1994)); yeast NBP35 (NBP35 protein, X95533 (Vitale *et al.*, 1996)); *Me. jannaschii* NBP (*Me. jannaschii* nucleotide-binding protein, U67483 (Bult *et al.*, 1996)); *E. coli* MRP (MRP\_ECOLI MRP protein, U00007 (Dardel *et al.*, 1990)); *H. influenzae* MRP (MRP\_HAEIN MRP protein homolog, U32807 (Fleischmann *et al.*, 1995)); *P. fragi* ATPase (*P. fragi* ATPase, U62986 (Michel *et al.*, 1997)); *Synechocystis* MRP (*Synechocystis* sp. MRP\_SYNY3 MRP protein homolog, D64001 (Kaneko *et al.*, 1995, 1996)); *B. subtilis* 38.5 KD (YBAL\_BACSU hypothetical 38.6 KD protein in CWLD-GERD intergenic region, X74737 (Sekiguchi *et al.*, 1995)); *My. leprae* MRP (*My. leprae* MRP protein homolog, U15180).

the EST entries from the IMAGE Consortium cDNA clones (Lennon *et al.*, 1996). Since the cDNA clone did not encode a complete protein coding sequence, a human teratocarcinoma cDNA library was screened by using this EST clone as a probe. We sequenced the entire 1.4-kb insert of the isolated cDNA clone (data now shown) and confirmed that this gene is indeed an ortholog of mouse *Nubp2* and lacks the idiosyncratic cysteine-rich N-terminal sequence of *Nubp1*. We named the human gene *NUBP2*.

#### Comparative Sequence Analyses of *Nubp1*, *Nubp2*, and Related Genes

The availability of sequence for mouse *Nubp1*, *Nubp2*, and human *NUBP2* permits a more extensive analysis of their relatedness and the phylogenetic relationships of other reported NUBP/MRP genes. A phylogenetic tree clearly discriminated two eukaryotic gene clusters: short form (*Nubp2*) and long form (*Nubp1*) (Fig. 2). The genes in one cluster from different organisms are more alike than are genes from the same organisms, but in the other cluster. For example, mouse *Nubp2* is more similar to human *NUBP2* than it is to mouse *Nubp1*. This phylogenetic tree also shows that yeast has two corresponding genes: *YIA3w* belongs to the *Nubp2* group, and *NBP35* belongs to the *Nubp1* group. We found only one *C. elegans* gene that

belongs to the *Nubp1* group in the GenBank and Sanger Center databases (<http://www.sanger.ac.uk>), but we predict that there will be another *C. elegans* gene that belongs to the *Nubp2* group. It is difficult to think that a gene that exists in yeast does not exist in *C. elegans*.

All the prokaryotic *mrp*-related genes show weak similarity to the eukaryotic *Nubp1*/*Nubp2* gene family in the phylogenetic tree (Fig. 2). Interestingly, the archaeobacteria *Me. jannaschii* NBP is more similar to the eukaryotic *Nubp1*/*Nubp2* genes than to the other prokaryotic *mrp*-related genes (Fig. 2). As reported in the genome sequence of this organism, *Me. jannaschii* can be classified between eukaryotes and prokaryotes based on sequence similarity analyses of many genes (Bult *et al.*, 1996). The NUBP gene analysis is consistent with this notion.

Figure 3 shows the alignment of all the corresponding amino acid sequences. The alignment was first performed with the Higgins algorithm on MacDNASIS, and gaps were then added manually to improve the alignment of motifs. At least four subregions of the proteins are strikingly conserved throughout prokaryotes and eukaryotes. The region nearest the N-terminal is the well-known ATP/GTP-binding motif A or P-loop (Walker *et al.*, 1982; Saraste *et al.*, 1990; Koonin, 1993), whose consensus is "[VI]-X-S-G-K-G-G-V-G-K-S-[TS]." A second region overlaps the ATP/GTP-

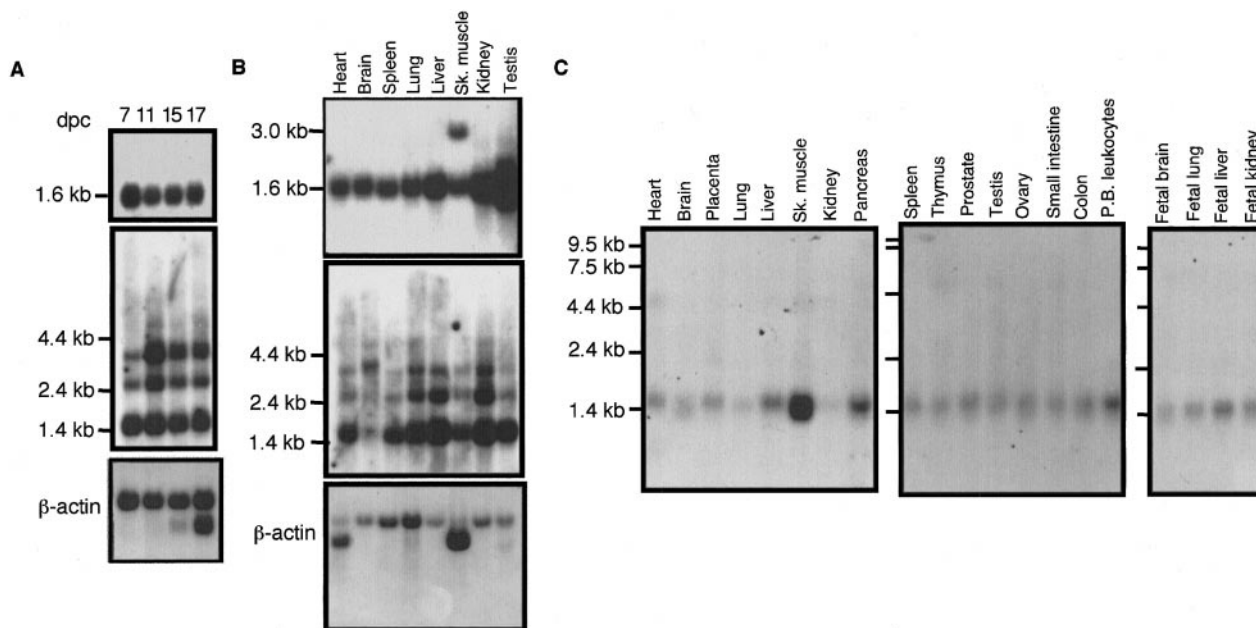
H_NUBP2	1	10	20	30	40	50	260	270	280	290	300
M_Nubp2	1	1	1	1	1	1	SISIKMSVF	LEFGDEAVY	WRGKPKALI	KQFSDVWAG	ELDIYAVL
Y_YIA3	1	1	1	1	1	1	SISIKMSVF	LEFGDEAVY	WRGKPKALI	KQFSDVWAG	ELDIYAVL
H_NBP	1	1	1	1	1	1	LSI	LEFGDEAVY	WRGKPKALI	KQFSDVWAG	ELDIYAVL
M_Nubp1	1	1	1	1	1	1	LSI	LEFGDEAVY	WRGKPKALI	KQFSDVWAG	ELDIYAVL
C_F10G8_6	1	1	1	1	1	1	LSI	LEFGDEAVY	WRGKPKALI	KQFSDVWAG	ELDIYAVL
Y_NBP35	1	1	1	1	1	1	LSI	LEFGDEAVY	WRGKPKALI	KQFSDVWAG	ELDIYAVL
M_NBP	1	1	1	1	1	1	LSI	LEFGDEAVY	WRGKPKALI	KQFSDVWAG	ELDIYAVL
SY_MRP	1	1	1	1	1	1	LSI	LEFGDEAVY	WRGKPKALI	KQFSDVWAG	ELDIYAVL
PF_ATPase	1	1	1	1	1	1	LSI	LEFGDEAVY	WRGKPKALI	KQFSDVWAG	ELDIYAVL
H_MRP	1	1	1	1	1	1	LSI	LEFGDEAVY	WRGKPKALI	KQFSDVWAG	ELDIYAVL
E_MRP	1	1	1	1	1	1	LSI	LEFGDEAVY	WRGKPKALI	KQFSDVWAG	ELDIYAVL
ML_MRP	1	1	1	1	1	1	LSI	LEFGDEAVY	WRGKPKALI	KQFSDVWAG	ELDIYAVL
BS_38_6KD	1	1	1	1	1	1	LSI	LEFGDEAVY	WRGKPKALI	KQFSDVWAG	ELDIYAVL

H_NUBP2	51	60	70	80	90	100	310	320	330	340	350
M_Nubp2	51	1	1	1	1	1	EGSDIRMAAT	IPALVYVOP	SAWLVTRP	AVSIVGVR	---
Y_YIA3	51	1	1	1	1	1	EGSDIRMAAT	IPALVYVOP	SAWLVTRP	AVSIVGVR	---
H_NBP	51	1	1	1	1	1	EGSDIRMAAT	IPALVYVOP	SAWLVTRP	AVSIVGVR	---
M_Nubp1	51	1	1	1	1	1	EGSDIRMAAT	IPALVYVOP	SAWLVTRP	AVSIVGVR	---
C_F10G8_6	51	1	1	1	1	1	EGSDIRMAAT	IPALVYVOP	SAWLVTRP	AVSIVGVR	---
Y_NBP35	51	1	1	1	1	1	EGSDIRMAAT	IPALVYVOP	SAWLVTRP	AVSIVGVR	---
M_NBP	51	1	1	1	1	1	EGSDIRMAAT	IPALVYVOP	SAWLVTRP	AVSIVGVR	---
SY_MRP	51	1	1	1	1	1	EGSDIRMAAT	IPALVYVOP	SAWLVTRP	AVSIVGVR	---
PF_ATPase	51	1	1	1	1	1	EGSDIRMAAT	IPALVYVOP	SAWLVTRP	AVSIVGVR	---
H_MRP	51	1	1	1	1	1	EGSDIRMAAT	IPALVYVOP	SAWLVTRP	AVSIVGVR	---
E_MRP	51	1	1	1	1	1	EGSDIRMAAT	IPALVYVOP	SAWLVTRP	AVSIVGVR	---
ML_MRP	51	1	1	1	1	1	EGSDIRMAAT	IPALVYVOP	SAWLVTRP	AVSIVGVR	---
BS_38_6KD	51	1	1	1	1	1	EGSDIRMAAT	IPALVYVOP	SAWLVTRP	AVSIVGVR	---

H_NUBP2	101	110	120	130	140	150	360	370	380	390	400
M_Nubp2	101	1	1	1	1	1	LITRGRSIT	RVMELVENMA	QVLSITENMS	SEVHITL	TSVYR
Y_YIA3	101	1	1	1	1	1	LITRGRSIT	RVMELVENMA	QVLSITENMS	SEVHITL	TSVYR
H_NBP	101	1	1	1	1	1	LITRGRSIT	RVMELVENMA	QVLSITENMS	SEVHITL	TSVYR
M_Nubp1	101	1	1	1	1	1	LITRGRSIT	RVMELVENMA	QVLSITENMS	SEVHITL	TSVYR
C_F10G8_6	101	1	1	1	1	1	LITRGRSIT	RVMELVENMA	QVLSITENMS	SEVHITL	TSVYR
Y_NBP35	101	1	1	1	1	1	LITRGRSIT	RVMELVENMA	QVLSITENMS	SEVHITL	TSVYR
M_NBP	101	1	1	1	1	1	LITRGRSIT	RVMELVENMA	QVLSITENMS	SEVHITL	TSVYR
SY_MRP	101	1	1	1	1	1	LITRGRSIT	RVMELVENMA	QVLSITENMS	SEVHITL	TSVYR
PF_ATPase	101	1	1	1	1	1	LITRGRSIT	RVMELVENMA	QVLSITENMS	SEVHITL	TSVYR
H_MRP	101	1	1	1	1	1	LITRGRSIT	RVMELVENMA	QVLSITENMS	SEVHITL	TSVYR
E_MRP	101	1	1	1	1	1	LITRGRSIT	RVMELVENMA	QVLSITENMS	SEVHITL	TSVYR
ML_MRP	101	1	1	1	1	1	LITRGRSIT	RVMELVENMA	QVLSITENMS	SEVHITL	TSVYR
BS_38_6KD	101	1	1	1	1	1	LITRGRSIT	RVMELVENMA	QVLSITENMS	SEVHITL	TSVYR

H_NUBP2	151	160	170	180	190	200	410	420	430	440	450
M_Nubp2	151	1	1	1	1	1	GEFTR	AGVFFG	VPDIDLMRT	LEE	HDF
Y_YIA3	151	1	1	1	1	1	GEFTR	AGVFFG	VPDIDLMRT	LEE	HDF
H_NBP	151	1	1	1	1	1	GEFTR	AGVFFG	VPDIDLMRT	LEE	HDF
M_Nubp1	151	1	1	1	1	1	GEFTR	AGVFFG	VPDIDLMRT	LEE	HDF
C_F10G8_6	151	1	1	1	1	1	GEFTR	AGVFFG	VPDIDLMRT	LEE	HDF
Y_NBP35	151	1	1	1	1	1	GEFTR	AGVFFG	VPDIDLMRT	LEE	HDF
M_NBP	151	1	1	1	1	1	GEFTR	AGVFFG	VPDIDLMRT	LEE	HDF
SY_MRP	151	1	1	1	1	1	GEFTR	AGVFFG	VPDIDLMRT	LEE	HDF
PF_ATPase	151	1	1	1	1	1	GEFTR	AGVFFG	VPDIDLMRT	LEE	HDF
H_MRP	151	1	1	1	1	1	GEFTR	AGVFFG	VPDIDLMRT	LEE	HDF
E_MRP	151	1	1	1	1	1	GEFTR	AGVFFG	VPDIDLMRT	LEE	HDF
ML_MRP	151	1	1	1	1	1	GEFTR	AGVFFG	VPDIDLMRT	LEE	HDF
BS_38_6KD	151	1	1	1	1	1	GEFTR	AGVFFG	VPDIDLMRT	LEE	HDF

H_NUBP2	201	210	220	230	240	250	460	470	480	490	500
M_Nubp2	201	1	1	1	1	1	ATNPAK	IL	DATPA	PA	---
Y_YIA3	201	1	1	1	1	1	ATNPAK	IL	DATPA	PA	---
H_NBP	201	1	1	1	1	1	ATNPAK	IL	DATPA	PA	---
M_Nubp1	201	1	1	1	1	1	ATNPAK	IL	DATPA	PA	---
C_F10G8_6	201	1	1	1	1	1	ATNPAK	IL	DATPA	PA	---
Y_NBP35	201	1	1	1	1	1	ATNPAK	IL	DATPA	PA	---
M_NBP	201	1	1	1	1	1	ATNPAK	IL	DATPA	PA	---
SY_MRP	201	1	1	1	1	1	ATNPAK	IL	DATPA	PA	---
PF_ATPase	201	1	1	1	1	1	ATNPAK	IL	DATPA	PA	---
H_MRP	201	1	1	1	1	1	ATNPAK	IL	DATPA	PA	---
E_MRP	201	1	1	1	1	1	ATNPAK	IL	DATPA	PA	---
ML_MRP	201	1	1	1	1	1	ATNPAK	IL	DATPA	PA	---
BS_38_6KD	201	1	1	1	1	1	ATNPAK	IL	DATPA	PA	---



**FIG. 4.** Northern blot analysis for mouse *Nubp2* mRNA (A, B), mouse *Nubp1* mRNA (A, B), and human *NUBP2* (C). Membranes containing staged mouse embryos (A) and various adult organs (B) were hybridized with the whole insert of clone C00001C09 as a probe for mouse *Nubp2* and IMAGE clone ID695233 as the probe for mouse *Nubp1*. (C) Northern analysis of expression of human *NUBP2* probed with the entire human *NUBP2* insert.

binding motif A' (Koonin, 1993), but the significant conservation can be extended much further in the N-terminal direction. The consensus sequence is "D-X-D-X-X-G." A third region is the one we call the NUBP/MRP motif  $\alpha$ . This region partially includes the Mrp family signature, which was originally developed for the PROSITE database (Bairoch *et al.*, 1997). They used an *E. coli* protein mrp that is a 41-kDa ATP-binding protein of unknown function as the prototype of this family. The consensus pattern was established as "W-X2-[LIVM]-D-[LIVMY]4-D-X-P-P-G-T-[GS]-D" (Bairoch *et al.*, 1997). With this new sequence alignment (Fig. 3), we propose that this conserved region can be extended in the N-terminal direction and covers 37 amino acid residues. The consensus pattern can now be defined as "[VI]-WRG-X5-[LIMA]-[LI]-X2-[FLM]-[VILF]-X4-W-X2-[LIVM]-D-[LIVMY]4-D-X-P-P-G-T-[GS]-D." A fourth region is the one we have named NUBP/MRP motif  $\beta$  and can be defined as "[VI]-[VIML]-G-[VIL]-[VI]-E-N-M-[SA]." Based on these four highly conserved motifs, we propose that this group of genes forms a distinctive NUBP/MRP subfamily.

In contrast to the previous classification as the partitioning ATPase gene family (Koonin, 1993) or MinD/MRP family (Voss *et al.*, 1995), we think that this NUBP/MRP subfamily is distinguishable from a closely related *MinD* gene family, for the following reasons. First, *E. coli* and *B. subtilis* *MinD* genes have a similar but somewhat different ATP/GTP-binding motif A, "S-G-K-G-G-V-G-K-T-T-T," and A', "D-X-D-I-

G." Second, *MinD* has only a portion of NUBP/MRP motif  $\alpha$ . Third, *MinD* does not have the NUBP/MRP motif  $\beta$ .

*Expression Analyses of Mouse Nubp1 and Nubp2 and Human NUBP2*

Southern blot analyses clearly find only one copy of both mouse *Nubp1* and *Nubp2* genes (data not shown). Northern blot analyses of the *Nubp2* gene showed a single transcript of 1.6 kb, consistent with the sequence analyses. The gene is expressed rather ubiquitously throughout embryogenesis (Fig. 4A). In adult organs, the highest expression of this gene was observed in testis, but the gene was also expressed in other organs (Fig. 4B). An additional 3.0-kb transcript observed in skeletal muscle suggests a possible alternatively spliced species.

In Northern blot analysis, a major 1.4-kb transcript of *Nubp1* was observed throughout development (Fig. 4A) and in many adult organs (Fig. 4B). Additional transcripts of 2.4 and 4.4-kb were also observed in most of the tissues (Figs. 4A and 4B), again suggesting the existence of alternatively spliced forms.

Northern blot analyses of human *NUBP2* showed ubiquitous expression in adult and fetal tissues, with highest expression in skeletal muscle of the adult. Once again, the 1.4-kb transcript is consistent with sequencing results (Fig. 4C). Taken together, the ubiq-

**FIG. 3.** Multiple alignment of protein sequences of the NUBP/MRP gene family. Identical amino acids are highlighted. Gaps are introduced to maximize the alignment. For sequence references see Fig. 2 legend. H\_NUBP2, human *NUBP2*; M\_Nubp2, mouse *Nubp2*; SY\_MRP, *Synechocystis* MRP.

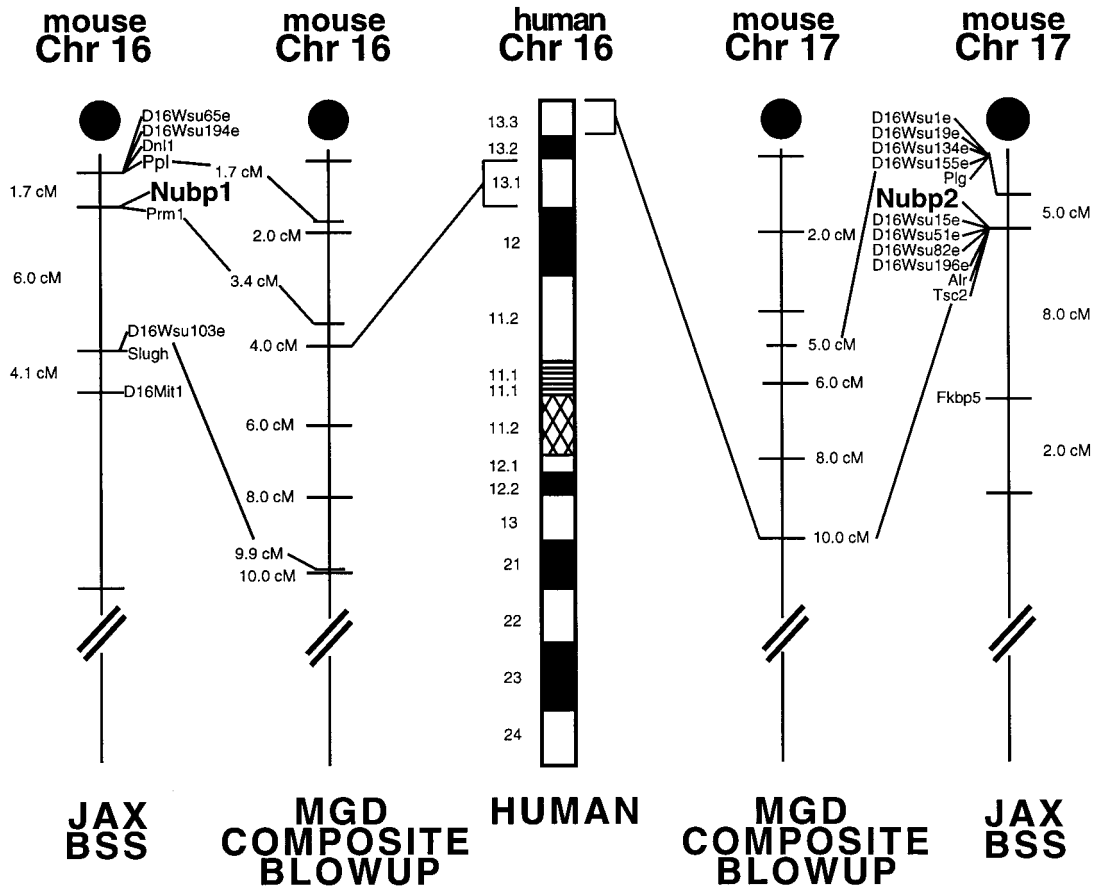


FIG. 5. Map location of mouse Nubp1 and mouse Nubp2.

uitous conservation and expression of the NUBP/MRP gene family suggest that the genes are essential.

#### Mapping of Mouse Nubp2 and Nubp1 on the Genetic Map

The *Nubp2* gene was originally mapped to the proximal region of Chromosome 17 as an EST (Ko *et al.*, 1998). Because the *Nubp1* sequence is very similar to the *Nubp2* sequence, it is highly likely that they stem from the same ancestral gene and evolved into two different genes by gene duplication and subsequent diversion (Ohno, 1970). Often such genes stay side by side until they are separated by the global evolution of chromosomes. Thus, we expected that *Nubp1* would map in the vicinity of *Nubp2*. Rather *Nubp1* mapped to the proximal region of Chromosome 16 (Fig. 5).

Based on the syntenic and homologous correlation between the human and mouse genomes (DeBry and Seldin, 1996), the human genomic DNA segment corresponding to the mouse *Nubp2* gene is chromosome 16p13.3 and that corresponding to mouse *Nubp1* gene is chromosome 16p13.1 (Fig. 5). Therefore, there will probably be a linkage conservation of human *NUBP2* and *NBP* (renamed *NUBP1*), and they may be juxtaposed, suggesting that a chromosomal breakage between *Nubp2* and *Nubp1* probably occurred during the evolution of mouse chromosomes.

#### Possible Function of NUBP/MRP Gene Family

Although the exact functions of Nubp1 and Nubp2 are yet to be elucidated, several lines of evidence give us reason to believe that both Nubp1 and Nubp2 play crucial roles. First, both genes are evolutionarily well conserved among eukaryotes. Second, both genes are rather ubiquitously expressed throughout development and adult organs. Third, it is shown that mutations and knockouts of the yeast gene *NBP35* were lethal (Vitale *et al.*, 1996). Preliminary analyses of the putative yeast *YIA3w* gene also show that knockout of the gene is lethal (R.M., work in progress). Because *Nubp1* and *Nubp2* show weak similarity to cell division inhibitors such as *CDI* of *Me. jannaschii* and *MinD* of *E. coli*, one possibility is that Nubp1/Nubp2 may be involved in the cell division process or cell cycle control. Perhaps more detailed analyses of yeast *NBP35* and *YIA3w* will provide a route to test this idea.

Is there a speculative rationale for the existence of both *Nubp1* and *Nubp2*? Their most significant difference is the presence or absence of the characteristic C-X13-C-X2-C-X5-C sequence in the N-terminal region of the genes. The prokaryotic NUBP/MRP family, like Nubp2, lacks this characteristic sequence, although *Me. Jannaschii* has a partially conserved version, C-X3-C-X2-C-X5-C. Perhaps the Nubp2 family is an ancestral form, and when eukaryotes developed a

membrane-bound nucleus, the Nubp1 family evolved to perform a similar function within the nucleus. Indeed, it has been shown with immunofluorescent tags that the yeast NBP35 protein (Nubp1 homolog) is observed mainly in the nucleus (Vitale *et al.*, 1996).

The mouse *t*-complex, where the mouse *Nubp2* is mapped, has drawn broad attention and has been one of the best studied regions of the mouse genome because of its many unusual and interesting features, including large genomic inversions and segregation distortions (meiotic drive) (Bennett, 1975; Silver, 1993). The region also contains many homozygous lethal mutant loci, which show dramatic phenotypes at an early stage of embryogenesis (Bennett, 1975). Another interesting feature of the *t*-complex is the presence of genes undergoing genomic imprinting—parent-of-origin-specific gene expression (Cattanach and Jones, 1994; Barlow, 1995; Leighton *et al.*, 1996). Because imprinted genes often exist as clusters on the genome, it is likely that the *t*-complex contains new imprinted genes in addition to the previously identified genes, *Igf2r* (Barlow *et al.*, 1991) and *Mas1* (Villar and Pedersen, 1994). Preliminary Southern blot analyses of the *Nubp2* gene with the methylation-sensitive *HpaII* enzyme have revealed that the region contains hemizygotously methylated sites, and thus, the gene may be a candidate for a new imprinted gene in the *t*-complex.

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