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The *ras*-related *ypt* protein is an ubiquitous eukaryotic protein: isolation and sequence analysis of mouse cDNA clones highly homologous to the yeast *YPT1* gene

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The *YPT1* gene of the yeast *Saccharomyces cerevisiae* codes for a guanine nucleotide-binding protein which is essential for cell viability. Using as hybridization probe cloned yeast *YPT1* gene sequences, we have isolated from cDNA libraries prepared from RNA of mouse F9 and C3H10T1/2 cells several overlapping cDNA clones with identical sequence in the regions of overlap. The cDNAs were derived from a gene, designated *ypt1*, which codes for a protein of 205 amino acids with 71% homology to the yeast *YPT1* gene product. Amino acid sequences typical for guanine nucleotide-binding proteins and characteristic for *ypt* proteins are perfectly conserved in the mouse *ypt1* protein. Two mRNAs of 1600 and 3200 nucleotides, originating from the mouse *ypt1* gene and differing in the length of their 3′-non-translated region, were identified in mouse F9 cells and in all mouse tissues examined. A monoclonal antibody specifically recognizing the 23.5-kd yeast *YPT1* protein cross-reacted with a protein of identical size on protein blots of mouse, rat, pig, bovine and human cell lines.

Key words: cDNA/guanine nucleotide binding/mouse/*ras*-related/*ypt1* protein

Introduction

The *ras*-related *YPT1* gene product was previously identified as an essential protein in the budding yeast *Saccharomyces cerevisiae* (Gallwitz *et al.*, 1983; Schmitt *et al.*, 1986a). The 206 amino acid long protein shares with the mammalian *ras* proteins very similar biochemical properties: it binds and hydrolyses guanine nucleotides specifically, it can be autophosphorylated as the result of an Ala→Thr substitution in a structurally identical domain required for nucleotide binding and hydrolysis (Wagner *et al.*, 1987) and it is substrate for palmitoylation (Molenaar and Gallwitz, unpublished results).

The homology of the yeast *YPT1* protein and the mammalian *ras* proteins is 38% in the region of the first 168 amino acid residues and is most pronounced within the five domains that seem to be critical for GTP binding and hydrolysis (Gallwitz *et al.*, 1983; Wagner *et al.*, 1987; for review, see Barbacid, 1987). As mutations of the *YPT1* protein that significantly impair nucleotide binding render the protein biologically inactive (Wagner *et al.*, 1987) it is clear that the reversible binding of GDP and GTP is essential for the functioning of the protein which, like the *ras* proteins and the classical G-proteins (for review, see Gilman, 1987), might be part of a signal transducing pathway.

The *ras* proteins that have been discovered first in mammals as products of a family of closely related genes, H-*ras* (Dhar *et al.*, 1982; Capon *et al.*, 1983), K-*ras* (Tsuchida *et al.*, 1982; Shimizu *et al.*, 1983; McGrath *et al.*, 1983), N-*ras* (Taparowsky *et al.*, 1983) and R-*ras* (Lowe *et al.*, 1987), are evolutionarily highly conserved and have been found also in the yeast *S. cerevisiae* (DeFeo-Jones *et al.*, 1983; Powers *et al.*, 1984) and *Schizosaccharomyces pombe* (Fukui and Kaziro, 1985), the fruit fly *Drosophila melanogaster* (Neuman-Silberberg *et al.*, 1984) and the slime mold *Dictyostelium discoideum* (Reymond *et al.*, 1984). Another group of proteins, designated *rho*, is distantly related to the *ras* proteins, and highly homologous members of this group, with all the known structural features of guanine nucleotide-binding proteins, have been identified in eukaryotic organisms as distant as human, molluscs (*Aplysia*) and yeast (Madaule and Axel, 1985; Madaule *et al.*, 1987).

Studies with yeast have clearly established that the protein products of the *RAS1* and *RAS2* genes (Tatchell *et al.*, 1984; Kataoka *et al.*, 1984), the *YPT1* gene (Schmitt *et al.*, 1986a, Segev and Botstein, 1987) and the *RHO1* gene (Madaule *et al.*, 1987) are essential for cell viability and, in spite of their structural relatedness, fulfill different biological functions.

We therefore initiated an investigation on the existence of *ypt* proteins in other eukaryotes and describe in this report the isolation and sequence analysis of cDNA clones from two different mouse cell lines whose protein product is highly homologous to the *S.cerevisiae YPT1* protein. We also show that the *ypt1* gene is expressed in a variety of mouse tissue.

Results

Isolation and sequences analysis of mouse ypt1 cDNA clones

DNA fragments of the protein coding region of cloned *YPT1* genes of the budding yeast *S.cerevisiae* (Gallwitz *et al.*, 1983)

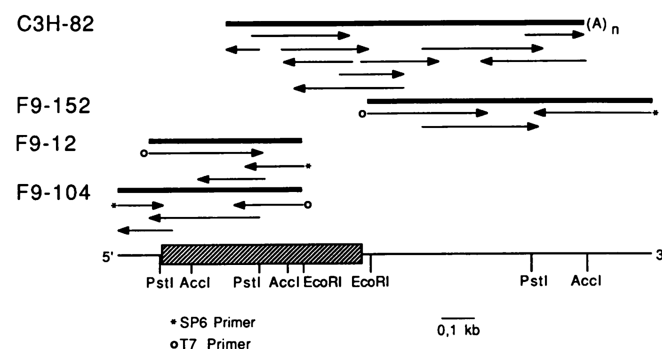

Fig. 1. Schematic representation of subcloned *Eco*RI fragments isolated from individual *ypt1* cDNA clones of the F9 cDNA library and the insert of the recombinant plasmid derived from the C3H10T1/2 cDNA library. The extent of nucleotide sequences of the overlapping fragments that were determined by plasmid sequencing using synthetic primers is indicated by arrows. Note that the cDNA fragment F9-152 goes past the polyadenylation site identified in clone C3H-82. The lower part of the figure shows the contiguous cDNA composed of sequences determined from the overlapping fragments. Also shown are restriction sites used to prepare fragments for RNA and DNA blot analyses. The boxed region represents the protein coding part.

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```
                             -100                            -70
TCGACGTTTGCTCTACCGGAACAGCTTAGCTCATTCCTCCCTTTCCATTACCTGTGGCGC

                              -40                            -10
GGAGAGTTGGGGCGGCGGCTGCGTCAGAAGGGCGGTGGTGGCGGCGGCGGCGGCGGCAGC

      -1
TGCAGTGACATGTCCAGCATGAATCCCGAATATGATTATTTATTCAAGTTACTTCTGATT
         MetSerSerMetAsnProGluTyrAspTyrLeuPheLysLeuLeuLeuIle
                                       10

GGCGATTCTGGGGTTGGAAAGTCCTGCCTTCTCCTTAGGTTTGCAGATGATACGTATACG
GlyAspSerGlyValGlyLysSerCysLeuLeuLeuArgPheAlaAspAspThrTyrThr
      20                           30

GAAAGCTACATCAGCACAATTGGTGTGGATTTCAAGATACGAACTATAGAGTTAGATGGG
GluSerTyrIleSerThrIleGlyValAspPheLysIleArgThrIleGluLeuAspGly
      40                           50

AAAACAATCAAGCTACAGATATGGGACACAGCAGGCCAGGAAAGATTTCGAACAATCACT
LysThrIleLysLeuGlnIleTrpAspThrAlaGlyGlnGluArgPheArgThrIleThr
      60                           70

TCCAGTTATTACAGAGGAGCCCATGGCATCATAGTTGTGTATGATGTGACAGATCAGGAG
SerSerTyrTyrArgGlyAlaHisGlyIleIleValValTyrAspValThrAspGlnGlu
      80                           90

TCCTTCAATAACGTTAAACAGTGGCTGCAGGAGATAGATCGCTACGCCAGTGAAAATGTC
SerPheAsnAsnValLysGlnTrpLeuGlnGluIleAspArgTyrAlaSerGluAsnVal
     100                          110

AACAAGTTGTTGGTAGGGAACAAATGTGACCTGACCACAAAGAAAGTAGTAGACTACACA
AsnLysLeuLeuValGlyAsnLysCysAspLeuThrThrLysLysValValAspTyrThr
     120                          130

ACAGCAAAGGAATTTGCAGATTCCCTTGGAATTCCATTTTTGGAAACCAGTGCTAAGAAC
ThrAlaLysGluPheAlaAspSerLeuGlyIleProPheLeuGluThrSerAlaLysAsn
     140                          150

GCAACGAATGTAGAACAGTCTTTCATGACGATGGCAGCTGAGATTAAAAAGCGAATGGGT
AlaThrAsnValGluGlnSerPheMetThrMetAlaAlaGluIleLysLysArgMetGly
     160                          170

CCTGGAGCTACAGCTGGTGGTGCCGAGAAGTCCAATGTTAAAATCCAGAGCACTCCAGTC
ProGlyAlaThrAlaGlyGlyAlaGluLysSerAsnValLysIleGlnSerThrProVal
     180                          190
                                                          -30
AAGCAGTCAGGTGGAGGCTGCTGCTAAAATCTGCCTCCGTCCTTTTCTCACAGCAATGAA
LysGlnSerGlyGlyGlyCysCysStp
     200           205
                                                          -90
TTCGCAATCTGAACCCAAGTGAAAAAACAAAATTGCCTGAATTGTACTGTATGTAGCTGC

                           -120                          -150
ACTACAACAGATTCTTACCGTTTCCACAAGGTCAGAGATTGTAAATGGTCAATACTGACT

                           -180                          -210
TTTTTTTTTATTCCCTTGACTCAAGACCGCTAACTTCATTTTCAGAACTGTTTTAAACCT

                           -240                          -270
TTGTGTGCTGGTTTATAAAATAATGTGTGTAATCCTTGTTGCTTTCCTGATACCAGATCG

                           -300                          -330
TTTCCCGTGGTTGGTTAGAATATATTTTGTTTTGATGTTTATATTGGCATGTTTAGATGT

                           -360                          -390
TGGGTTTAGTCTTCTGAAGATGAAGTTCAGCCATTTTGTATCACACAGCACAACCAGTGT

                           -420                          -450
CTGTCAGTTTCCACGCATAAAGTTTAGTGAGACGTTATATGTAAGATCTGATTTGCTAGT

                           -480                          -510
TCTTCCTGGTAGAGTTATAAATGGAAAGATTACACTATCTGATTAATAGTTTCTTCATAC

                           -540                          -570
TCTGCATATAATTTGTGGCTGCAGAATATTGTAATTTGTTGCACACTATGTAACAAAACT

                           -600                          -630
GAAGATATGTTTAATAAATATTGTACTTATTGGAAGTAATATCAAACTGTATGGTGATAA

                           -660              -681
GTATTGTTTTAATTCCTATGATTAAAGGGAAATAGAGCTTTGCATTCTAAAAAAAAAAAA

AAAAAAAAA
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Fig. 2. Nucleotide sequence and deduced amino acid sequence of the mouse *yptl* cDNA. The sequence was derived from fragments of individual cDNA clones (see Figure 1) isolated from an F9 and a C3H10T1/2 library. The exact 5′ end of the *yptl* mRNA is not known. The 3′ end of the sequence shown, including the poly(A) tail, was derived from clone C3H-82. The sequence of 219 nucleotides of clone F9-152, extending further 3′ of the polyadenylation site identified with clone C3H-82, is not shown. Putative polyadenylation signal sequences are underlined. Amino acids are numbered beginning with the first methionine codon of the open reading frame.

```
                                                    *
mouse ypt1    1   M S S M N P E Y D Y L F K L L L I G D S G V G K S
s.cer YPT1    1         M N S E Y D Y L F K L L L I G N S G V G K S

mouse ypt1   26   C L L L R F A D D T Y T E S Y I S T I G V D F K I
s.cer YPT1   23   C L L L R F S D D T Y T N D Y I S T I G V D F K I

mouse ypt1   51   R T I E L D G K T I K L Q I W D T A G Q E R F R T
s.cer YPT1   48   K T V E L D G K T V K L Q I W D T A G Q E R F R T

mouse ypt1   76   I T S S Y Y R G A H G I I V V Y D V T D Q E S F N
s.cer YPT1   73   I T S S Y Y R G S H G I I I V Y D V T D Q E S F N

mouse ypt1  101   N V K Q W L Q E I D R Y A S E N V N K L L V G N K
s.cer YPT1   98   G V K M W L Q E I D R Y A T S T V L K L L V G N K

mouse ypt1  126   C D L T T K K V V D Y T T A K E F A D S L G I P F
s.cer YPT1  123   C D L K D K R V V E Y D V A K E F A D A N K M P F

mouse ypt1  151   L E T S A K N A T N V E Q S F M T M A A E I K K R
s.cer YPT1  148   L E T S A L D S T N V E D A F L T M A R Q I K E S

mouse ypt1  176   M G P G A T A G G A E K S N V K I Q S T P V K Q S
s.cer YPT1  173   M S Q Q N L N E T T Q K K E D K G N V N L K G Q S

                              * *
mouse ypt1  201           G G G C C
s.cer YPT1  198   L T N T G G G C C
```

Fig. 3. Comparison of amino acid sequences of the *S.cerevisiae YPT1* gene product and the mouse *yptl* protein deduced from the cDNA sequence. Identical residues are boxed. Dots between pairs of residues indicate favoured substitution (Dayhoff *et al.*, 1978). Residues characteristic for *ypt* proteins in comparison with *ras* and *rho* proteins are marked with asterisks (see text).

and the fission yeast *S.pombe* (Haubruck *et al.*, unpublished data) were labelled with [^{32}P]dCTP using random primers and used to screen a cDNA library prepared in phage λgt10 from RNA of retinoic acid-induced F9 cells. Using moderately stringent hybridization conditions (4 × SSC and 60°C) several cross-hybridizing recombinant phages were identified, purified and cut with the restriction endonuclease *Eco*RI. The resulting restriction fragments were subcloned into *Eco*RI-cut plasmid pSPT18 and subjected to plasmid sequence analysis using T7 and SP6 sequencing primers.

As can be seen in Figure 1, two overlapping fragments containing parts of the protein-coding and the 5′-untranslated region and having identical sequence in the regions of overlap were identified. The cDNA clones F9-104 and F9-12 contained 129 and 37 nucleotides, respectively, of 5′-untranslated region and 445 nucleotides of the protein-coding sequence. Comparison of the protein sequence deduced from the mouse cDNA sequences with that of the primary structure of the *S.cerevisiae YPT1* protein (Gallwitz *et al.*, 1983) revealed a surprisingly high degree of homology. The sequences of the yeast and the mouse YPT protein could be perfectly aligned and of the first 148 amino acid residues that could be compared 81% were identical. Assuming the first AUG codon of the only open reading frame to be the translation initiation codon, the mouse *yptl* protein would be longer by three residues at the N terminus (see Figures 2 and 3).

As we had not found a full-length cDNA clone in the collection of F9 clones isolated, we screened a cDNA library constructed in the vector pcD (Okayama and Berg, 1983) from RNA of a 3-methylcholantrene-treated C3H10T1/2 mouse fibroblast cell line (Shih *et al.*, 1979) using a 325-bp *Pst*I fragment of the coding part of the mouse cDNA clone F9-12 (see Figure 1). Under stringent hybridizing conditions (2 × SSC, 68°C) two positive clones out of ~150 000 clones screened were identified and purified. The recombinant plasmids pcD-YPT82 and pcD-YPT71 carried inserts of ~1270 bp and 1050 bp, respectively. The larger of the two cDNAs, designated C3H-82 (see Figure 1), was subjected to plasmid sequence analysis using several primer oligonucleotides that were synthesized according to the

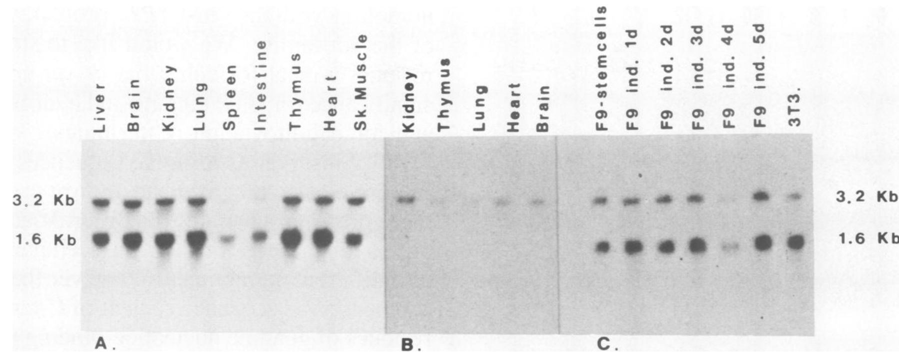

Fig. 4. Northern blot analysis of RNA derived from different mouse tissues (**A** and **B**), from mouse 3T3 fibroblasts and from F9 cells induced to differentiate into parietal endoderm with 5×10^{-7} M retinoic acid and 10^{-3} M dibutyryl cAMP for 1–5 days (**C**). RNA blots shown in **A** and **C** were probed with a labelled 325-bp *Pst*I fragment of the *ypt1* protein-coding region; the blot presented in **B** was hybridyzed with a 218-bp *Acc*I fragment of the 3'-non-translated region of cDNA F9-152 which is beyond the polyadenylation site found in the cDNA C3H-82 (see Figure 1). Approximately 10 μg of total RNA was separated on each lane.

sequences established from F9 cDNA clones described above and the C3H-82 cDNA itself. As can be seen in Figures 1 and 2, the cDNA C3H-82 terminated with a poly(A) tail 23 nucleotides downstream of the presumptive polyadenylation signal sequence ATTAAA. A second polyadenylation signal sequence (AATA-AA) was located 69 nucleotides further upstream. Although the cDNA C3H-82 was not of full length, the overlap of 218 nucleotides with the cDNA F9-12 and F9-104 was identical to the nucleotide and it is therefore most likely that the cDNAs from the F9 and the C3H10T1/2 cell libraries originated from the same mRNA. This is supported by the fact that an *Eco*RI fragment of 870 bp subcloned from another recombinant phage of the F9 cDNA library (F9-152) had an identical sequence to cDNA C3H-82 over a region of 651 nucleotides beginning with the *Eco*RI recognition sequence 31 nucleotides downstream of the translation stop codon UAA and ending exactly at the start of the poly(A) tail (see Figures 1 and 2). The cDNA F9-152, however, extended the 3' end of the C3H-82 cDNA by 219 nucleotides but it did not terminate with a poly(A) tail. This suggests that at least two mRNAs are generated from the mouse *ypt1* gene, the smaller of which should have a minimal length of 1428 nucleotides without poly(A) tail.

Mouse and yeast YPT proteins are highly homologous

A comparison of the amino acid sequences of the *S.cerevisiae YPT1* gene product (206 residues) and the mouse *ypt1* protein deduced from the cDNA sequence is given in Figure 3. With the exception of four residues (amino acids in position 198–201 of the *S.cerevisiae YPT1* protein) the sequences could be perfectly aligned. The overall homology of the two proteins is remarkable. 71% of the amino acid residues are identical and, including conservative exchanges, the homology is 83.5%. The homology with 87% identities (or 95% including conservative exchanges) is most impressive in the region of the first 125 amino acids. The homology abruptly ends at residue 170 (with respect to the yeast protein), but the C-terminal five residues, Gly-Gly-Gly-Cys-Cys, are again identical. The domains that have been shown to be of importance for guanine nucleotide binding of mammalian *ras* proteins (for review, see Barbacid, 1987) and for the *S.cerevisiae YPT1* protein (Wagner *et al.*, 1987) are also conserved in the mouse *ypt1* gene product. Residues in two functionally significant regions of mammalian *ras* proteins and the yeast *YPT1* protein that we consider to be characteristic for *ypt* proteins, a serine residue instead of glycine-12 (*ras*) and two consecutive cysteine residues instead of a cysteine residue followed by two aliphatic and another amino acid (*ras*) at the C-terminal end, are found in both the yeast and the mouse protein.

Expression of the ypt1 gene in different mouse tissues

The expression of the mouse *ypt1* gene was investigated by Northern blot analysis of RNA isolated from various mouse tissues and from uninduced and retinoic acid-induced F9 teratocarcinoma cells. As shown in Figure 4A–C, in all mouse tissues and in F9 cells two RNA species of ~1600 and 3200 nucleotides were detected using as hybridization probe either the 325-bp *Pst*I fragment of the protein-coding region or the 501-bp *Eco*RI/*Pst*I fragment of the 3'-non-translated region (see Figure 1). The smaller of the two transcripts fits the minimal length of the mRNA predicted from the cDNA sequence very well [1428 nucleotides without poly(A) tail]. The 3200-nucleotide RNA but not the 1600-nucleotide RNA hybridysed to a 216-bp *Acc*I fragment, the 3'-non-translated region of the F9-152 cDNA clone, downstream of the polyadenylation site of the C3H-82 cDNA clone. This is evidence for the two mRNAs being derived from the same gene but differing in the length of their 3'-non-translated regions.

The relative amounts of these two RNA species as well as the total amounts of *ypt1* mRNAs seemed not to be significantly different in the mouse tissues examined and in F9 cells differentiating into parietal endoderm.

A monoclonal antibody against the yeast YPT1 protein cross-reacts with a 23-kd protein of several mammalian cell lines

The *S.cerevisiae YPT1* gene product and the mouse *ypt1* protein with 206 and 205 amino acid residues have nearly identical molecular mass. Among the different antibodies prepared against the yeast *YPT1* protein, a monoclonal antibody (Y-27B1) specifically recognizing the yeast protein (Figure 5B) was used to search for the presence of cross-reacting proteins in mouse and other mammalian cell lines.

Total cellular protein of the different cell lines of mouse, rat, pig, bovine and human origin was separated by SDS–PAGE, transferred to nitrocellulose filters and challenged with the supernatant of the hybridoma cell secreting the monoclonal antibody Y-27B1. As can be seen in Figure 5A, a 23-kd protein having the same mobility as the yeast *YPT1* protein was clearly identified in all cell lines. The antibody reacted specifically with the

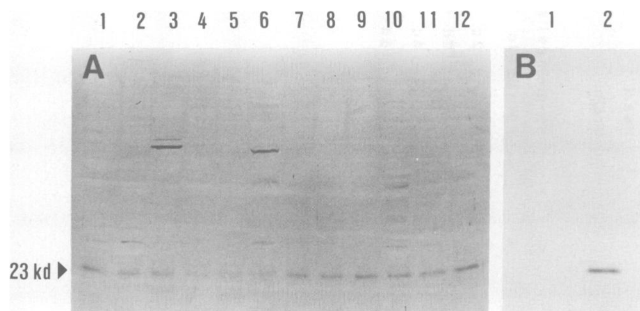

Fig. 5. Identification of mammalian and yeast proteins cross-reacting with a monoclonal antibody directed against the yeast *YPT1* gene product. **(A)** Total protein of a human glioma cell line (**1**), LLCPK1 pig kidney cells (**2**), RMC rat memory cells (**3**), A375 human melanoma cells (**4**), MDBK bovine kidney cells (**5**), A431-7 ATCC human epidermal cells (**6**), HeLa cells (**7**), mouse SW3T3 fibroblasts (**8**), HS27 human fibroblasts (**9**), MCF7-13 human breast carcinoma cells (**10**), RD·X human rhabdomyosarcoma cells (**11**) and human adrenal cortex cells (**12**) or **(B)** total protein of *E.coli JM101* expressing the v-K-*ras* protein from the gene on an expression vector (**1**) or the *S.cerevisiae* cells overexpressing the *YPT1* gene on a yeast expression vector (Schmitt *et al.*, 1986a) (**2**), was separated by SDS–PAGE, transferred electrophoretically to nitrocellulose filters and treated with a monoclonal antibody directed against the yeast *YPT1* protein. Cross-reacting proteins were identified with sheep anti-mouse Ig linked to horseradish peroxidase. Note that the *YPT1*-specific antibody does not cross-react with either the v-K-*ras* protein expressed in *E.coli* (**B, lane 1**) or the p21 *ras*-gene products of the different mammalian cell lines. Other cross-reacting proteins of some cell lines are of unknown nature.

yeast *YPT1* protein and did not cross-react with the v-K-*ras* protein produced in *Escherichia coli* (Figure 5B) or the p21 *ras* gene products of the mammalian cell lines tested (Figure 5A).

Discussion

The different overlapping *ypt1* cDNA clones with identical sequence that we have isolated from cDNA libraries of mouse F9 and C3H10T1/2 cells are most likely copies of two mRNAs derived from the same gene. This is supported by the finding that the two mRNA species found in all mouse tissues examined could be identified on RNA blots with different hybridizing DNA fragments of the protein-coding and the 3′-non-translated region whereas the larger of the two mRNAs hybridized only with DNA sequences past the polyadenylation site identified in the C3H-82 cDNA clone.

The primary structure of the mouse protein deduced from the cDNA sequence can be perfectly aligned with the amino acid sequence of the *S.cerevisiae YPT1* gene product, and the high degree of sequence homology, 87% of identical residues within the region of the first 125 amino acids and 71% of identities when the total sequences are compared, show that the mouse *ypt1* protein and the yeast *YPT1* gene product are members of a family of closely related guanine nucleotide-binding proteins distinct from *ras* and *rho* proteins. On the basis of additional sequence information of *ypt* homologues isolated from the fission yeast *S.pombe* (our unpublished data) and from the slime mold *D.discoideum* (A.Kimmel, personal communication) we conclude that certain structural features are diagnositc for *ypt* proteins: among other characteristics they have a serine instead of a glycine residue found in position 12 of normal *ras* proteins and, in contrast to *ras* and *rho* proteins whose C terminus is a cysteine followed by three other residues, *ypt* proteins terminate with two cysteines (see Figures 2 and 3). According to this classification, the recently identified secretion-required *SEC4* gene product of *S.cerevisiae* (Salminen and Novick, 1987), which shares 47% homology with the yeast *YPT1* protein, would also be a member of the *ypt* family. We would like to stress that the amino acid residues typical for either *ras* or *ypt* proteins are of functional significance for GTP binding and hydrolysis (N-terminal region) and for palmitoylation (C terminus). It could well be that the highly conserved C-terminal sequences that distinguish *ras* and *rho* proteins on the one hand and *ypt* proteins on the other make these proteins substrates for different acylating enzymes and/or are of importance for their intracellular sorting and integration into different membranes. Whatever the meaning of the distinct and conserved sequence context of C-terminally located cysteine residues of guanine nucleotide-binding proteins might be, it has been well documented for mammalian *ras* proteins (Willumsen *et al.*, 1984; Chen *et al.*, 1985; Buss and Sefton, 1986) and yeast *RAS* proteins (Fujiyama and Tamanoi, 1986; Powers *et al.*, 1986;. Deschenes and Broach, 1987) as well as for the *S.cerevisiae YPT1* protein (Molenaar and Gallwitz, unpublished data) that palmitic acid becomes covalently attached to these cysteine residues and that this modification is of importance for the biological function of the proteins.

We have shown previously that yeast cells deficient in *YPT1* protein or those expressing a mutant *YPT1* protein resulting in dominant lethality are defective in mitosis and display severe cytoskeletal lesions (Schmitt *et al.*, 1986a). Segev and Botstein (1987) using a cold-sensitive yeast *ypt1* mutant gene came to a similar conclusion and in addition showed that the *YPT1* gene product is also required for sporulation and the response to starvation. Our recent studies show that yeast cells depleted of *YPT1* protein and cells carrying a temperature-sensitive *ypt1* allele at the non-permissive temperature accumulate membranous structures, most likely endoplasmic reticulum, but that all these defects might be secondary to a disregulation of intracellular calcium (H.D.Schmitt *et al.*, in preparation).

YPT proteins seem to serve basic cellular functions in eukaryotes. It was therefore not surprising to observe that the *ypt1* gene is expressed in all mouse tissues and cell lines examined. The demonstration in rat, pig, bovine and human cells of a protein of identical size to the yeast *YPT1* protein that cross-reacted with an antibody directed against the yeast protein is proof of the ubiquity of *ypt* proteins in eukaryotic cells.

We will have to demonstrate whether or not the mouse *ypt1* protein serves a function similar to the *YPT1* protein in yeast. Experiments in progress in which the protein-coding part of the *S.cerevisiae YPT1* gene is being replaced by that of the mouse *ypt1* gene are expected to give at least an indication as to whether these two proteins are integrated in similar regulatory pathways in yeast and mammalian cells.

Materials and methods

cDNA library screening

About 150 000 λgt10 plaques of a cDNA library prepared from RNA of retinoic acid-induced F9 cells (Schmitt *et al.*, 1986b) were screened with ^{32}P-labelled DNA fragments of the protein-coding region of the cloned *YPT1* gene of *S.cerevisiae* (Gallwitz *et al.*, 1983) and the *ypt1* gene of *S.pombe* (Haubruck *et al.*, unpublished data). Fifteen positive clones were found by hybridization at 60°C in 4 × SSC. Recombinant phages were purified and inserted DNA fragments hybridizing to the yeast DNA probe were cut out with *Eco*RI, subcloned in the plasmid pSPT18 (Pharmacia) and subjected to plasmid sequencing (Chen and Seeburg, 1985) using SP6 and T7 sequencing primers and other synthetic primers synthesized according to sequences established. The 326-bp *Pst*I fragment of the coding region of the cDNA F9-104 (see Figure 1) was then used to screen a cDNA library prepared from RNA of a transformed C3H10T1/2 cell line (kindly provided by H.Okayama). At a hybridization temperature of 68°C in 2 × SSC, two positive clones were found among 150 000 clones tested. Hybridization solutions were prepared according to Maniatis *et al.* (1982).

RNA analysis

Total cellular RNA was isolated from F9 cells and from different tissues of adult mice using the guanidinium thiocyanate/CsCl method of Chirgwin *et al.* (1979). RNA was denatured by glyoxylation (McMaster and Carmichael, 1977) and aliquots of 10 μg were separated on a 1.2% agarose gel and transferred onto gene screen plus. Hybridizations using DNA fragments labelled with random primers (Feinberg and Vogelstein, 1983) were performed at 45°C for 20 h in 0.2 M sodium phosphate, pH 7.2, 1 mM EDTA, 1% bovine serum albumin, 7% SDS and 45% formamide. Filters were washed twice in 40 mM sodium phosphate, pH 7.2, and 1% SDS for 10 min at room temperature and then at 65°C for 15 min in the same solution.

Immunoblot analysis

Total cellular protein was separated on 12.5% polyacrylamide–SDS gels (Laemmli, 1970) and transferred to nitrocellulose filters as described (Schmitt *et al.*, 1986a). Protein blots were treated with the monoclonal antibody Y-27B1 directed against the yeast *YPT1* protein and stained with peroxidase-conjugated sheep anti-mouse Ig (Amersham) according to the supplier's recommendations.

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