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The *ras*-related mouse ypt1 protein can functionally replace the *YPT1* gene product in yeast

Heinz Haubruck, Reinhild Prange[1], Constantin Vorgias and Dieter Gallwitz

Max-Planck-Institut for Biophysical Chemistry, Department of Molecular Genetics, PO Box 2841, D-3400 Göttingen, FRG

[1]Present address: Institute of Medical Microbiology, University of Mainz, FRG

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The protein-coding region of the essential *Saccharomyces cerevisiae YPT1* gene coding for a *ras*-related, guanine-nucleotide-binding protein was exchanged in chromosome VI by the protein-coding segment of either the mouse *ypt1* gene or the v-Ki-*ras* gene, and different chimeric *YPT1*–v-Ki-*ras* genes. The mouse ypt1 protein with 71% of identical residues compared with the yeast Ypt1 protein could functionally fully replace its yeast homologue as long as the mouse gene was overexpressed under transcriptional control of the inducible *GAL10* promoter. In contrast, neither the viral Ki-*ras* nor the hybrid proteins were able to substitute for the loss of *YPT1* gene function. This study suggests that different parts of the yeast Ypt1 protein are required for the interaction with cellular targets and that these essential parts are conserved in the mammalian ypt1 protein.

Key words: guanine nucleotide binding/mouse/ras-homologue/*Saccharomyces cerevisiae*/yeast/ypt1 protein

Introduction

Besides the G proteins, membrane-associated, guanine-nucleotide-binding, regulatory proteins composed of three subunits (for review see Gilman, 1987), eukaryotic cells contain a variety of other structurally related proteins that function as monomers in different regulatory pathways through their capacity to bind and hydrolyse GTP specifically. Of these proteins, the p21 products of the Ha-, Ki- and N-*ras* genes (for review see Barbacid, 1987) have attracted greatest attention as mutated versions can cause malignant transformation of mammalian cells.

The ypt proteins, a family of ubiquitous eukaryotic proteins (Haubruck *et al.*, 1987), are structurally related to the *ras* gene products and share with them very similar biochemical properties (Wagner *et al.*, 1987). In the yeast *Saccharomyces cerevisiae*, where this class of proteins was first discovered (Gallwitz *et al.*, 1983), the *YPT1* gene product serves an essential function (Schmitt *et al.*, 1986; Segev and Botstein, 1987). Mutations of the protein that affect its capacity for GPT binding (Schmitt *et al.*, 1986; Wagner *et al.*, 1987) or membrane association (Molenaar *et al.*, 1988) are lethal. Genetic and biochemical studies have clearly shown that, in yeast, Ras and Ypt1 proteins are functionally not interchangeable; whereas the *RAS1* and *RAS2* gene products modulate adenylyl cyclase activity (Toda *et al.*, 1985), the Ypt1 protein seems to be involved in protein secretion and/or the regulation of intracellular calcium (Schmitt *et al.*, 1988; Segev *et al.*, 1988).

The comparison of primary sequences of ras and ypt proteins reveals a clustering of identical residues in four regions which, by mutational analysis, have been shown to take part in guanine nucleotide binding and hydrolysis [for reviews see Wagner *et al.* (1987) and Barbacid (1987)]. These regions are located within the first 160 residues and are followed by some 30–40 residues of rather variable sequence. The yeast *RAS*-gene-encoded proteins have even 150–160 residue-long C-terminal extensions of unrelated sequence (DeFeo-Jones *et al.*, 1983; Powers *et al.*, 1984). Nevertheless, the human Ha-*ras* gene can substitute, at least in part, for the loss of *RAS* gene function in yeast (DeFeo-Jones *et al.*, 1985; Kataoka *et al.*, 1985).

One way of identifying functionally important regions in structurally related proteins is to probe for biological activity of hybrid proteins generated by exchanging defined segments between these related proteins. Such an approach was made by studying, after chromosomal integration, chimeric genes generated between the viral Ki-*ras* and the yeast *YPT1* gene. The v-Ki-*ras* gene was chosen for this study as its p21 product contains a Ser instead of a Gly residue in position 12, the corresponding position being likewise occupied by Ser in ypt proteins of different species. In ras proteins, the substitution of Gly-12 for Ser results in a decrease of GTPase activity and concomitantly confers upon the mutant proteins transforming activity (Gibbs *et al.*, 1984; McGrath *et al.*, 1984; Manne *et al.*, 1985). Another way of studying the structure/function relationship of proteins exhibiting sequence homology is to probe for their functional interchangeability.

We show in this report that the mouse ypt1 protein exhibiting >70% of sequence identity with its yeast counterpart (Haubruck *et al.*, 1987) is indeed able to functionally replace the yeast Ypt1 protein, whereas chimeric proteins comprising the v-Ki-*ras* and the yeast Ypt1 protein are non-functional in yeast.

Results

The mouse ypt1 protein is biologically active in yeast

In contrast to the mammalian ras proteins with <40% of identical residues with respect to the yeast Ypt1 protein, the recently discovered ypt1 protein of mouse shares 71% of identical sequence with its yeast homologue (Haubruck *et al.*, 1987). We engineered the mouse *ypt1* cDNA such that newly created *Nde*I and *Bss*HII restriction sites allowed the exact exchange of the protein-coding regions of both genes (Figure 1). Gene replacement by homologous recombination was achieved by transforming either a haploid or a diploid yeast strain with a linear DNA fragment carrying the mouse *ypt1*-, instead of the yeast *YPT1*-coding region and the *LEU2* selectable marker gene followed by the bacterial plasmid pUC8. These additional sequences that had been in-

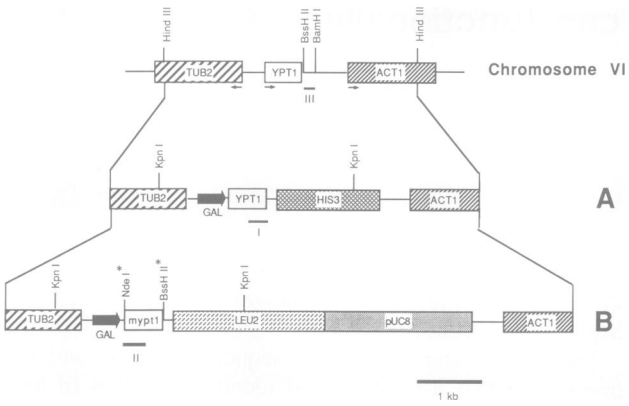

Fig. 1. Introduction of *GAL10*-controlled yeast *YPT1* and mouse *ypt1* genes into the *S.cerevisiae* chromosome VI. The localization of the *YPT1* gene on chromosome VI between the β-tubulin and the actin gene and relevant restriction sites are shown in the upper part of the figure. The direction of transcription of the three genes is indicated by arrows. (**A**) Strain HLR3 (see Materials and methods) carries the *YPT1* gene under *GAL10* promoter control and contains the *HIS3* gene as selectable marker, inserted into the *Bam*HI restriction site. This strain was used to analyse growth properties (Figure 4) and to exchange the yeast *YPT1* gene by the *GAL10*-controlled mouse *ypt1* gene using the linearized eviction vector pEV-GALmypt1. (**B**) Successful transformants became his3$^-$ and Leu2$^+$. Restriction sites introduced at the 5′ and 3′ end of the mouse *ypt1*-coding region are indicated by asterisks. DNA fragments used as hybridization probes in RNA blot analyses are labelled I–III.

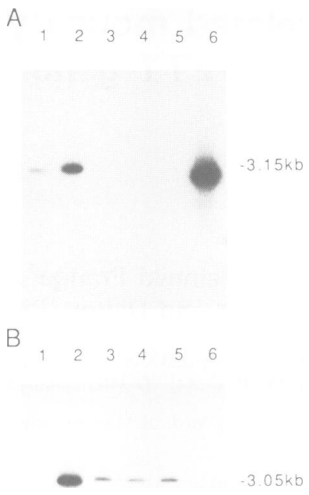

Fig. 2. Southern blot analysis to show correct gene replacement in yeast transformants. *S.cerevisiae* haploid strain HLR3 carrying the *YPT1* gene under *GAL10* promoter control and the *HIS3* gene 3′ of the *YPT1* gene (**A** in Figure 1) was transformed with the *Hin*dIII-linearized vector pEV-GALmypt1 (**B** in Figure 1). Leu2$^+$ transformants were selected and 10 μg each of total DNA from four transformants were digested with *Kpn*I and subjected to Southern analysis using as hybridization probes (Figure 1) either fragment I specific for the yeast *YPT1* gene (**A**) or fragment II specific for the mouse *ypt1*-coding region (**B**). **Lane 1**, haploid strain HLR3 before transformation; **lane 2**, transformant of His3$^+$ Leu2$^+$ phenotype; **lanes 3–5**, Leu2$^+$ his3$^-$ transformants with correct gene replacement; **lane 6**, *Kpn*I-cut vector pEV-ScYPT1 generating a 3.05 kb fragment containing the yeast *YPT1* gene (to show probe specificity).

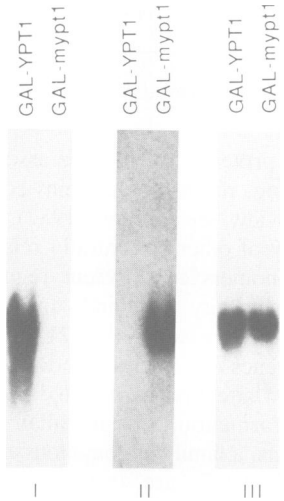

Fig. 3. RNA blot analysis of haploid strains expressing either the *GAL10* promoter-controlled yeast *YPT1* gene (GAL-YPT1) or the mouse *ypt1*-coding region (GAL-mypt1). About 10 μg of glyoxylated total cellular RNA from logarithmically growing cells were subjected to agarose gel electrophoresis and, after transfer to nitrocellulose, hybridized to probes specific for either the yeast *YPT1*-coding region (I, see also Figure 1), the mouse *ypt1* coding segment (II) or the 3′ untranslated region common to both transcripts (III).

serted into the *Bam*HI restriction site located 3′ of the *YPT1* gene (Figure 1) made possible the easy recovery of the genes introduced into the chromosome by cutting whole chromosomal DNA of transformants with *Hin*dIII and cloning the religated DNA in *Escherichia coli*.

As the haploid strain used for transformation carried the only functional *HIS3* gene 3′ to the *YPT1* gene on chromosome VI (see Figure 1) and the diploid strain used had the *YPT1* gene on one chromosome replaced by the *HIS3* gene, successful gene replacement should result in a his3$^-$ Leu2$^+$ phenotype. Viable haploid transformants were only obtained when the mouse *ypt1*-coding region was under transcriptional control of the strong *GAL10* promoter but not with a yeast *YPT1* promoter-controlled mouse gene. Diploid transformants with one *YPT1* gene replaced by the *YPT1*-controlled mouse homologue segregated 2:2 with respect to viability. To prove successful gene replacement, Southern analysis was performed with *Kpn*I-digested total DNA of three haploid transformants with the desired phenotype, and from one of the very rare transformants being His3$^+$ and Leu2$^+$. As expected, in the transformants being his3$^-$ Leu2$^+$ only the mouse *ypt1* sequences were observed (Figure 2, lanes 3–5), whereas the transformant of His3$^+$ and Leu2$^+$ phenotype harboured both yeast *YPT1* and mouse *ypt1* sequences resulting from an unwanted integration event (Figure 2, lane 2).

We also analysed on Northern blots total cellular RNA from haploid transformants carrying either the yeast *YPT1* gene or the mouse *ypt1* gene under the control of the *GAL10* promoter. Using hybridization probes specific for either the yeast or the mouse protein-coding region (Figure 1, probes I and II), the expected signals of the 800-nucleotide mRNAs were obtained (Figure 3). Most importantly, a hybridization probe of the 3′ untranslated region common to both mRNAs (Figure 1, probe III) gave signals of similar intensity when RNA from cells expressing the yeast *YPT1* or the mouse *ypt1* gene were compared.

Having established that the mouse ypt1 protein is indeed

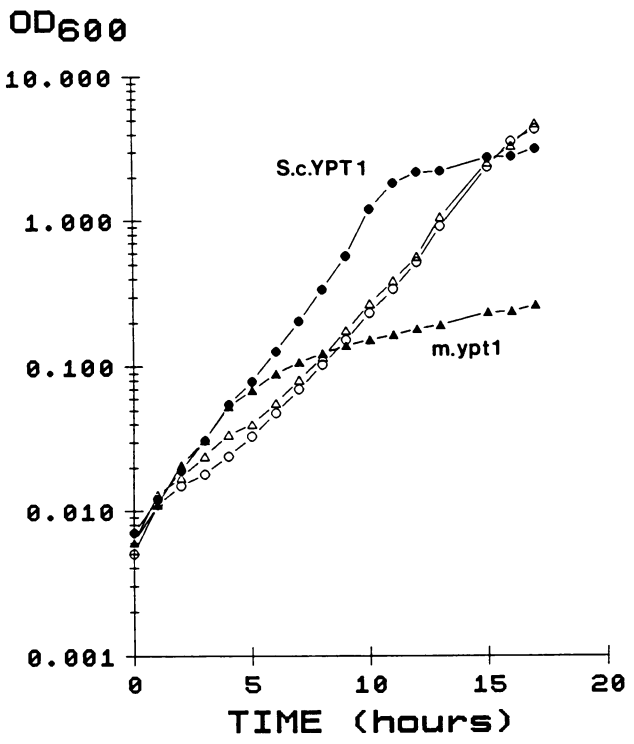

Fig. 4. Growth properties of haploid cells expressing the *S.cerevisiae* Ypt1 or the mouse ypt1 protein under *GAL10* promoter control. Cultures of logarithmically growing cells in galactose-containing medium were divided and one part of each culture was diluted with fresh medium to the indicated optical density for further growth in galactose (open symbols). To block the expression of the *GAL10*-controlled genes, cells of the other halves of the two cultures were pelleted by centrifugation, washed in pre-warmed, glucose-containing medium and allowed further growth in glucose (filled symbols). Samples were taken at 1 h intervals to determine the optical density at 600 nm. Note the somewhat longer generation time of cells grown in galactose (~2 h) as opposed to growth in glucose-containing medium (~90 min).

a functional homologue of the yeast Ypt1 protein and that yeast cells totally dependent on the mouse gene have the requirement for an enhanced expression of the foreign protein, we further enquired into the need for overexpression of the mouse ypt1 protein. We made use of the ability to shut off the *GAL10*-controlled gene, by transferring logarithmically growing cells to glucose-containing medium. Our previous studies (Schmitt *et al.*, 1986) had shown that switching off *YPT1* gene expression leads to a complete halt of cell division after several generation times, i.e. after ~10–12 h.

As can be seen in Figure 4, haploid strains with either the wild-type *YPT1* or the mouse *ypt1* protein-coding region under transcriptional control of the *GAL10* promoter exhibited comparable growth properties. However, after glucose-induced blocking of transcription, it took a significantly shorter time to inhibit the multiplication of cells relying on the mouse ypt1 protein (~5 h) than those depleted of the yeast Ypt1 protein (~12 h). Given a generation time of ~90 min of wild-type cells in glucose-containing medium, the growth curve of cells depleted of mouse ypt1 protein levelled off 4–5 generation times earlier than the growth curve of cells with the silenced yeast *YPT1* gene. As shown by RNA-blot analysis (Figure 3), the steady-state levels of mRNAs coding for either the mouse ypt1 or the

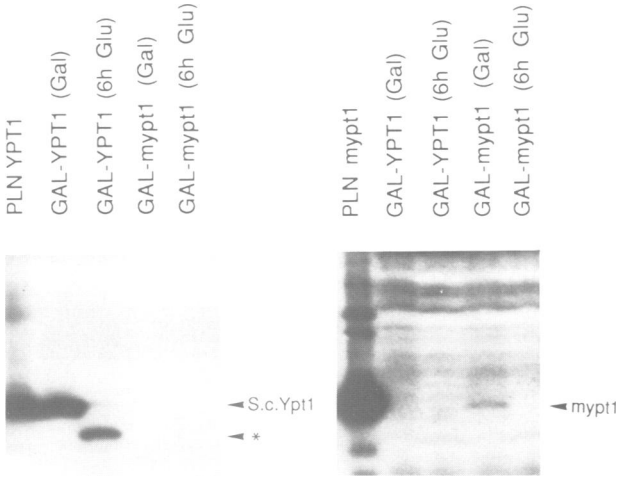

Fig. 5. Immunoblot analysis of total protein from *E.coli* (PLNYPT1 and PLNmypt1) and from haploid yeasts expressing either the *S.cerevisiae YPT1* or the mouse *ypt1* gene under transcriptional control of the *GAL10* promoter. Yeast cells were either grown logarithmically in galactose-containing medium (Gal) or transferred to glucose-containing medium for 6 h (Glu) to switch off the expression of the *GAL10*-controlled genes. Blots were treated with either a monoclonal antibody directed against and specific for the yeast Ypt1 protein (left part of the figure) or an affinity-purified polyclonal antibody directed against the mouse ypt1 protein (right part of the figure), followed by either a ^{125}I-labelled sheep anti-mouse or sheep anti-rabbit second antibody. Note the significantly lower level of mouse ypt1 versus yeast Ypt1 protein in logarithmically growing yeast cells and the degradation product (indicated by an asterisk) of the yeast Ypt1 protein seen 6 h after the shut-down of *YPT1* gene transcription.

yeast Ypt1 protein were very similar, excluding the possibility of a significant difference in mRNA stability.

This result suggests that the mouse ypt1 protein was relatively unstable in yeast cells. To test this assumption, steady-state levels of the ypt1 proteins expressed from *GAL10*-controlled genes were determined in haploid transformants on galactose-containing medium (logarithmic growth) and in cells 6 h after a shift to glucose-containing medium. Immunoblot analysis was performed with antibodies specific for either the yeast or the mouse protein. Similar amounts of bacterially produced ypt1 proteins gave signals of comparable intensity with iodine-labelled second antibodies. As is evident from the results shown in Figure 5, the amount of yeast Ypt1 protein was significantly larger than that of mouse ypt1 protein in the respective logarithmically growing transformants. Indeed, to detect the mouse protein in yeast, the blots had to be exposed to the X-ray film for a significantly longer period of time than those showing the yeast Ypt1 protein. Six hours after silencing the *GAL10*-controlled genes, yeast protein with the correct length was still detectable, but a larger amount of a degradation product, still able to bind guanine nucleotides (data not shown), appeared. In contrast, no trace of ypt1 protein could be detected under these conditions in cells harbouring the mouse protein-coding region (Figure 5).

The viral Ki-ras protein and chimeras of the v-Ki-ras and the yeast Ypt1 protein are non-functional in yeast

A sequence comparison of the v-Ki-ras and the yeast Ypt1 proteins shows 42% identity within the N-terminal 62 amino acids (residues 1–62 of the v-Ki-ras protein and residues 6–68 of the Ypt1 protein) and 35% of identical residues

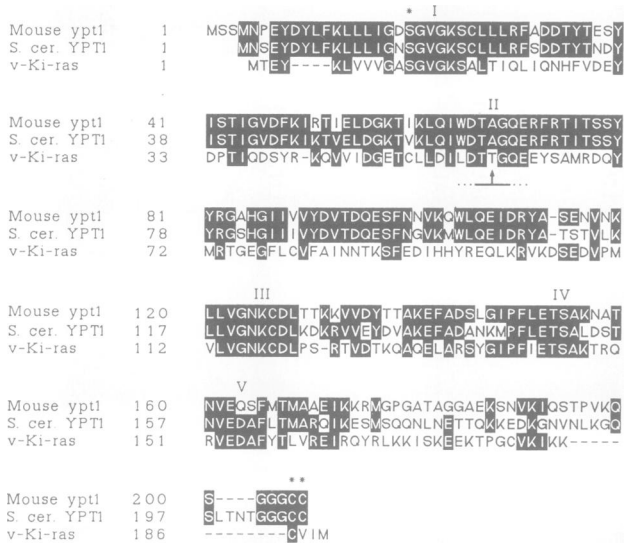

Fig. 6. Comparison of the amino acid sequences of the v-Ki-ras (Tsuchida *et al.*, 1982), the *S.cerevisiae* Ypt1 (Gallwitz *et al.*, 1983) and the mouse ypt1 proteins (Haubruck *et al.*, 1987). Identical residues are boxed. The segments of the v-Ki-*ras* protein-coding region (after mutating codon 59 to an Ala codon) and the yeast *YPT1*-coding region that were fused to generate the hybrids HYBI and HYBII (see text) are indicated by an arrow. The regions known from mutational analyses to be required for guanine nucleotide binding of ras proteins (for review see Barbacid, 1987) and the Ypt1 protein (Schmitt *et al.*, 1988; Wagner *et al.*, 1987) are marked I–V. Indicated with asterisks are structural features typical for ypt proteins; Ser in position 12 with respect to mammalian ras proteins and two Cys residues at the C-terminal end.

in the following 103 amino acids (Figure 6). Including preferred substitutions (Dayhoff *et al.*, 1978), the degree of homology within these two protein regions amounts to 64 and 58% respectively. The sequences beyond residue 165 (with respect to the ras protein) are not related at all. Sequence features typical for ypt proteins are a Ser residue instead of Gly found in position 12 of all cellular ras proteins and two consecutive Cys residues at the C-terminal end in contrast to the sequence Cys-XXX with which ras proteins terminate (Gallwitz *et al.*, 1983; Haubruck *et al.*, 1987).

Since the functionally important Gly residue in position 12 is mutated to Ser in the v-Ki-ras protein (Tsuchida *et al.*, 1982), we sought to examine whether this protein, or an engineered version terminating (like the Ypt1 protein) with two Cys residues, could substitute for the loss of the essential *YPT1* gene function. With a strategy similar to that outlined above, gene replacements were performed and it was found that neither of the two genes controlled either by the *YPT1* or the *GAL10* promoter, could functionally replace the *YPT1* gene, although the *ras* gene products were easily identified in yeast transformants by immunoblotting and by their GTP binding capacity (data not shown).

Similarly, hybrid proteins consisting of the N-terminal third of the v-Ki-ras protein (residues 1–59, see Figure 6) and the following two-thirds of the yeast Ypt1 protein (residues 66–206), HYBI, as well as the converse construction (HYBII), were unable to complement the loss of *YPT1* gene function. Although mRNAs of the two hybrid genes were observed in yeast transformants, the HYBII protein and the wild-type Ypt1 protein were present in comparable amounts in heterozygous diploids whereas the HYBI protein was very unstable in yeast and could not be identified at all (data not shown).

Discussion

The most significant result of our present study, aimed to get inside the structure/function relationship of the *ras*-related ypt proteins, is that the mouse ypt1 protein can functionally replace its yeast counterpart. The regions critical for recognizing specific cellular targets, therefore, seem to be highly conserved from yeast to mammals. The most plausible explanation for the increased expression of the mouse *ypt1* protein required to sustain yeast viability is its relative instability in the foreign environment. This is reflected by the markedly faster inhibition of cell proliferation following the shut-down of transcription of the *GAL10*-controlled mouse *ypt1*- as opposed to the yeast *YPT1*-coding sequence. Since the half-lives of yeast mRNAs are in the order of minutes and the steady-state levels of mRNAs harbouring the protein-coding regions of either the mouse *ypt1* or the yeast *YPT1* gene with identical 5′ and 3′ untranslated sequences were comparable, it seems highly unlikely that mRNA instability demanded the overexpression of the mouse sequence. The relative abundance of yeast Ypt1 protein compared with mouse ypt1 protein observed in the respective haploid transformants under identical conditions (*GAL10* promoter control, logarithmic growth) would also argue for a high instability of the mouse protein produced in yeast. A translational inefficiency of the mouse sequence, however, cannot be excluded.

The lethal effect observed after chromosomal exchange of the yeast *YPT1* by the v-Ki-ras protein-coding region again stresses the functional dissimilarity of these structurally related proteins. The failure of the viral ras protein to substitute for the loss of Ypt1 protein function is not simply explained by ras protein instability or lack of its GPT-binding ability since the protein itself and its nucleotide-binding activity were clearly demonstrated on protein blots of total yeast protein. As two structural features that are typical for ypt proteins, Ser in position 12 (with respect to ras proteins) and two consecutive Cys residues at the C-terminal end (Gallwitz *et al.*, 1983; Haubruck *et al.*, 1987), were retained in a mutant v-Ki-ras protein also found to be unfunctional, it seems reasonable to assume that regions required for interaction with cellular target proteins (receptor, effector) differ markedly in the two GTP-binding proteins. Indeed, a putative effector-binding region of ras proteins, residues 32–40 (Sigal *et al.*, 1986; Calés *et al.*, 1988; Adari *et al.*, 1988) being exposed on the outside of the molecules (De Vos *et al.*, 1988), has no obvious similarity to the corresponding region in the yeast Ypt1 and the mouse ypt1 protein (Figure 6). This region, however, is highly conserved between ypt proteins. If this part of ypt proteins was to serve a target-binding function, the incompetence of the HYBII protein in yeast would have a partial explanation. However, as this particular segment was retained in the chimeric HYBI protein that was also inactive, sequences in both parts of the Ypt1 protein used for hybrid generation (Figure 6) seem to be critical for its biological function. We cannot exclude, however, the possibility that the lack of HYBI protein function was simply due to its instability.

Chimeric proteins formed between defined segments of mammalian and *Drosophila melanogaster ras* gene products

on the one hand (Schejter and Shilo, 1985) and R-ras and Ha-ras proteins on the other (Lowe *et al.*, 1988) are transformation-competent. From the lack of transforming activity of the R-ras protein and certain R-*ras*−Ha-*ras* hybrids (Lowe and Goeddel, 1987; Lowe *et al.*, 1988), Lowe *et al.* (1988) concluded that several sequence segments of the folded protein might determine its biological activity. This seems also to apply to the *ypt* gene products as evidenced by the lack of function of the chimeras we have studied. The failure of the chimeric proteins to substitute for the loss of yeast *YPT1* gene function could also be the result of improper membrane targetting.

Considering the expression of the mouse *ypt1* gene in all the mouse tissues and cell lines examined (Haubruck *et al.*, 1987), its protein product is also likely to serve a very basic function in mammalian cells. The structural and functional homology of this guanine nucleotide-binding protein with the essential yeast Ypt1 protein further supports the notion that fundamental eukaryotic mechanisms, like signal transduction (DeFeo-Jones *et al.*, 1985; Kataoka *et al.*, 1985; and this report), cell cycle control (Lee and Nurse, 1987) or transcription regulation (Bohmann *et al.*, 1987; Metzger *et al.*, 1988; Schena and Yamamoto, 1988; Struhl, 1988) employ elements that are highly conserved in evolution.

Materials and methods

Oligonucleotide-directed mutagenesis and molecular cloning

Chemically synthesized oligonucleotides were used to introduce point mutations into cloned DNA following either a two-primer mutagenesis protocol (Zoller and Smith, 1982; Pielak *et al.*, 1985) or according to Nakamaye and Eckstein (1986).

To generate fragments for the replacement of the yeast *YPT1*- by the mouse *ypt1*-coding region, *Nde*I and *Bss*HII restriction sites were introduced at the 5′ and the 3′ end respectively, of the mouse protein-coding sequence. A 325 bp *Pst*I fragment of cDNA clone F9-12 harbouring the coding segment for the N-terminal half of the mouse ypt1 protein (Haubruck *et al.*, 1987) was cloned in phage M13mp19 and the *Nde*I restriction site was introduced with the oligonucleotide 5′-GCAGTGACATGTCCCATA-TGAATCCCGAATATG-3′. The *ypt1* cDNA clone C3H-82 (Haubruck *et al.*, 1987) as a 1275 bp *Bam*HI fragment was likewise cloned into phage M13mp19, and a *Bss*HII restriction site was generated with the oligonucleotide 5′-GCTGTGAGAAAAGGACGCGCGCAGATTTTAGCA-GCC-3′. From the two recombinant phages, a 245 bp *Nde*I−*Nco*I fragment and a 380 bp *Nco*I−*Bss*HII fragment containing the entire mouse *ypt1* protein-coding region, were used to construct, in several steps, the final eviction vectors pEV-mypt1 and pEV-GALmypt1 used for gene replacement (Figure 1).

Yeast genetic techniques

The diploid yeast strain DAH2215 (MAT*a*/MATα *YPT1/YPT1 leu2/leu2 his3/+ his4/+*) was used for complementation analyses with *ras* and hybrid genes. For the functional analysis of the mouse *ypt1* gene controlled by the yeast *YPT1* promoter, the diploid strain DAH430H (*MATa/MAT*α *YPT1/ypt1*:: *HIS3 leu2/leu2 his3/his3*) (Schmitt *et al.*, 1988) was transformed with *Hin*dIII-linearized vector pEV-mypt1. Of 40 transformants tested, 37 were of his3$^-$ phenotype. Four of these transformants were subjected to tetrad analysis and found to segregate 2:2 with respect to viability. The mouse *ypt1* gene under *GAL10* promoter control was introduced into chromosome VI by transforming the haploid strain HLR3 (*MATa GAL10-YPT1-HIS3 his3 leu2*) (Schmitt *et al.*, 1988) with the linearized plasmid pEV-GALmypt1. Of 30 clones tested, 29 were of Leu2$^+$his3$^-$ phenotype and showed the correct insertion of the mouse *ypt1* gene by Southern analysis. Yeast transformation was performed according to Hinnen *et al.* (1987) or by the method of alkali cation treatment (Ito *et al.*, 1983). Procedures for growth of yeast cells, sporulation of diploids, dissection of tetrads and scoring of genetic markers were carried out by standard methods (Mortimer and Hawthorne, 1969). For galactose inducible expression, yeast transformants were propagated in SD-medium containing 4% galactose instead of glucose.

Immunoblot analysis

For immunoblots, total extracts from ~2 × 10^8 bacteria or 2 × 10^7 yeast cells were taken up in detergent-containing buffer according to Laemmli (1970), boiled for 5 min and subjected to SDS−PAGE. Immunoblot analysis of the separated proteins was performed as described (Schmitt *et al.*, 1986), using either affinity-purified antibodies against the mouse ypt1 protein raised in rabbits or a monoclonal antibody (Y-27B10) directed against the yeast Ypt1 protein.

Other methods

Preparation of DNA and RNA, Northern and Southern blots and nucleotide-binding analysis were as described previously (Langford and Gallwitz, 1983; Langford *et al.*, 1984; Schmitt *et al.*, 1986; Wagner *et al.*, 1987).

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References

Adari,H., Lowy,D.R., Willumsen,B.M., Der,C.J. and McCormick,F. (1988) *Science*, **240**, 518−521.
Barbacid,M. (1987) *Annu. Rev. Biochem.*, **56**, 779−827.
Bohmann,D., Bos,T.J., Admon,A., Nishimura,T., Vogt,P.K. and Tjian,R. (1987) *Science*, **238**, 1386−1392.
Calés,C., Hancock,J.H., Marshall,C.J. and Hall,A. (1988) *Nature*, **332**, 548−551.
Dayhoff,M.O., Schwartz,R.M. and Orcutt,B.C. (1978) In Dayhoff,M.O. (ed.), *Atlas of Protein Sequence and Structure*. National Biomedical Research Foundation, Silver Spring, MD, Vol. 5, suppl. 3, pp. 345−352.
DeFeo-Jones,D., Scolnick,E.M., Koller,R. and Dhar,R. (1983) *Nature*, **306**, 707−709.
DeFeo-Jones,D., Tatchell,K., Robinson,L.C., Sigal,I.S., Vass,W.C., Lowy,D.R. and Scolnick,E.M. (1985) *Science*, **228**, 179−184.
De Vos,A.M., Tong,L., Milburn,M.V., Matias,P.M., Jangarik,J., Noguchi,S., Nishimura,S., Miura,K., Ohtsuka,E. and Kim,S.-H. (1988) *Science*, **239**, 888−893.
Gallwitz,D., Donath,C. and Sander,C. (1983) *Nature*, **306**, 704−707.
Gibbs,J.B., Sigal,I.S., Poe,M. and Scolnick,E.M. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 5704−5708.
Gilman,A.G. (1987) *Annu. Rev. Biochem.*, **56**, 615−649.
Haubruck,H., Disela,C., Wagner,P. and Gallwitz,D. (1987) *EMBO J.*, **6**, 4049−4053.
Hinnen,A., Hicks,J.B. and Fink,G.R. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 1929−1933.
Ito,H., Fukuda,Y., Murata,K. and Kimura,A. (1983) *J. Bacteriol.*, **153**, 163−168.
Kataoka,T., Powers,S., Cameron,S., Fasano,O., Goldfarb,M., Broach,J. and Wigler,M. (1985) *Cell*, **40**, 19−26.
Laemmli,U.K. (1970) *Nature*, **227**, 680−685.
Langford,C.J. and Gallwitz,D. (1983) *Cell*, **33**, 519−527.
Langford,C.J., Klinz,F.-J., Donath,C. and Gallwitz,D. (1984) *Cell*, **36**, 645−653.
Lee,M.G. and Nurse,P. (1987) *Nature*, **327**, 31−35.
Lowe,D.G. and Goeddel,D.V. (1987) *Mol. Cell. Biol.*, **7**, 2845−2856.
Lowe,D.G., Ricketts,M., Levinson,A.D. and Goeddel,D.V. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 1015−1019.
McGrath,J.P., Capon,D.J., Goeddel,D.V. and Levinson,A.D. (1984) *Nature*, **310**, 644−649.
Manne,V., Bekesi,E. and Kung,H.-F. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 376−380.
Metzger,D., White,J.H. and Chambon,P. (1988) *Nature*, **334**, 31−36.
Molenaar,C.M.T., Prange,R. and Gallwitz,D. (1988) *EMBO J.*, **7**, 971−976.
Mortimer,R.K. and Hawthorne,D.C. (1969) In Rose,A.H. and Harrison,J.S. (eds), *The Yeasts*. Academic Press, New York.
Nakamaye,K.L. and Eckstein,F. (1986) *Nucleic Acids Res.*, **14**, 9679−9698.
Pielak,G.J., Mauk,G.A. and Smith,M. (1985) *Nature*, **313**, 152−153.
Powers,S., Kataoka,T., Fasano,O., Goldfarb,M., Strathern,J., Broach,J. and Wigler,M. (1984) *Cell*, **36**, 607−612.
Schejter,E.D. and Shilo,B.-Z. (1985) *EMBO J.*, **4**, 407−412.

Schena,M. and Yamamoto,K.R. (1988) *Science*, **241**, 965–967.
Schmitt,H.D., Wagner,P., Pfaff,E. and Gallwitz,D. (1986) *Cell*, **47**, 401–412.
Schmitt,H.D., Puzicha,M. and Gallwitz,D. (1988) *Cell*, **53**, 635–647.
Segev,N. and Botstein,D. (1987) *Mol. Cell. Biol.*, **7**, 2367–2377.
Segev,N., Mulholland,J. and Botstein,D. (1988) *Cell*, **52**, 915–924.
Sigal,I.S., Gibbs,J.B., D'Alonzo,J.S. and Scolnick,E.M. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 4725–4729.
Struhl,K. (1988) *Nature*, **332**, 649–650.
Toda,T., Uno,I., Ishikawa,T., Powers,S., Kataoka,T., Broek,D., Cameron,S., Broach,J., Matsumoto,K. and Wigler,M. (1985) *Cell*, **40**, 27–36.
Tsuchida,N., Ryder,T. and Ohtsubo,E. (1982) *Science*, **217**, 937–939.
Wagner,P., Molenaar,C.M.T., Rauh,A.J.G., Bröckel,R., Schmitt,H.D. and Gallwitz,D. (1987) *EMBO J.*, **6**, 2373–2379.
Zoller,M.J. and Smith,M. (1982) *Nucleic Acids Res.*, **10**, 6487–6500.

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